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(54) Title: ALTERATIONS IN THE LONG QT SYNDROME GENES *KVLQT1* AND *SCN5A* AND METHODS FOR DETECTING SAME

(57) Abstract: Long QT Syndrome (LQTS) is a cardiovascular disorder characterized by prolongation of the QT interval on electrocardiogram and presence of syncope, seizures and sudden death. Five genes have been implicated in Romano-Ward syndrome, the autosomal dominant form of LQTS. These genes are *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* and *KCNE2*. Mutations in *KVLQT1* and *KCNE1* also cause the Jervell and Lange-Nielsen syndrome, a form of LQTS associated with deafness, a phenotypic abnormality inherited in an autosomal recessive fashion. Mutational analyses were used to screen 262 unrelated individuals with LQTS for mutations in the five defined genes. A total of 134 mutations were observed of which eighty were novel.

TITLE OF THE INVENTION

ALTERATIONS IN THE LONG QT SYNDROME GENES *KVLQT1* AND *SCN5A* AND METHODS FOR DETECTING SAME

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BACKGROUND OF THE INVENTION

Long QT Syndrome (LQTS) is a cardiovascular disorder characterized by prolongation of the QT interval on electrocardiogram and presence of syncope, seizures and sudden death, usually in young, otherwise healthy individuals (Jervell and Lange-Nielsen, 1957; Romano et al., 1963; Ward, 1964). The clinical features of LQTS result from episodic ventricular tachyarrhythmias, such as *torsade de pointes* and ventricular fibrillation (Schwartz et al., 1975; Moss et al., 1991). Two inherited forms of LQTS exist. The more common form, Romano-Ward syndrome (RW), is not associated with other phenotypic abnormalities and is inherited as an autosomal dominant trait with variable penetrance (Roman et al., 1963; Ward, 1964). Jervell and Lange-Nielsen syndrome (JLN) is characterized by the presence of deafness, a phenotypic abnormality inherited as an autosomal recessive trait (Jervell and Lange-Nielsen, 1957). LQTS can also be acquired, usually as a result of pharmacologic therapy.

In previous studies, we mapped LQTS loci to chromosomes 11p15.5 (*LQT1*) (Keating et al., 1991), 7 q35-36 (*LQT2*) (Jiang et al., 1994) and *LQT3* to 3p21-24 (Jiang et al., 1994). A fourth locus (*LQT4*) was mapped to 4q25-27 (Schott et al., 1995). Five genes have been implicated in Romano-Ward syndrome, the autosomal dominant form of LQTS. These genes are *KVLQT1* (*LQT1*) (Wang Q. et al., 1996a), *HERG* (*LQT2*) (Curran et al., 1995), *SCN5A* (*LQT3*) (Wang et al., 1995a), and two genes located at 21q22 - *KCNE1* (*LQT5*) (Splawski et al., 1997a) and *KCNE2* (*LQT6*) (Abbott et al., 1999). Mutations in *KVLQT1* and *KCNE1* also cause the Jervell and Lange-Nielsen syndrome, a form of LQTS associated with deafness, a phenotypic abnormality inherited in an autosomal recessive fashion.

KVLQT1, *HERG*, *KCNE1* and *KCNE2* encode potassium channel subunits. Four KVLQT1 α-subunits assemble with minK (β-subunits encoded by *KCNE1*, stoichiometry is

unknown) to form I_{Ks} channels underlying the slowly activating delayed rectifier potassium current in the heart (Sanguinetti et al., 1996a; Barhanin et al., 1996). Four HERG α-subunits assemble with MiRP1 (encoded by *KCNE2*, stoichiometry unknown) to form I_{Kr} channels, which underlie the rapidly activating, delayed rectifier potassium current (Abbott et al., 1999). Mutant subunits lead to reduction of I_{Ks} or I_{Kr} by a loss-of-function mechanism, often with a dominant-negative effect (Chouabe et al., 1997; Shalaby et al., 1997; Wollnik et al., 1997; Sanguinetti et al., 1996b). *SCN5A* encodes the cardiac sodium channel that is responsible for I_{Na}, the sodium current in the heart (Gellens et al., 1992). LQTS-associated mutations in *SCN5A* cause a gain-of-function (Bennett et al., 1995; Dumaine et al., 1996). In the heart, reduced I_{Ks} or I_{Kr} or increased I_{Na} leads to prolongation of the cardiac action potential, lengthening of the QT interval and increased risk of arrhythmia. *KVLQT1* and *KCNE1* are also expressed in the inner ear (Neyroud et al., 1997; Vetter et al., 1996). Others and we demonstrated that complete loss of I_{Ks} causes the severe cardiac phenotype and deafness in JLN (Neyroud et al., 1997; Splawski et al., 1997b; Tyson et al., 1997; Schulze-Bahr et al., 1997).

Presymptomatic diagnosis of LQTS is currently based on prolongation of the QT interval on electrocardiogram. Genetic studies, however, have shown that diagnosis based solely on electrocardiogram is neither sensitive nor specific (Vincent et al., 1992; Priori et al., 1999). Genetic screening using mutational analysis can improve presymptomatic diagnosis. However, a comprehensive study identifying and cataloging all LQTS-associated mutations in all five genes has not been achieved. To determine the relative frequency of mutations in each gene, facilitate presymptomatic diagnosis and enable genotype-phenotype studies, we screened a pool of 262 unrelated individuals with LQTS for mutations in the five defined genes. The results of these studies are presented in the Examples below.

The present invention relates to alterations in the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* and *KCNE2* genes and methods for detecting such alterations.

The publications and other materials used herein to illuminate the background of the invention or provide additional details respecting the practice, are incorporated by reference, and for convenience are respectively grouped in the appended List of References.

The present invention is directed to alterations in genes and gene products associated with long QT syndrome and to a process for the diagnosis and prevention of LQTS. LQTS is

diagnosed in accordance with the present invention by analyzing the DNA sequence of the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* gene of an individual to be tested and comparing the respective DNA sequence to the known DNA sequence of the normal gene. Alternatively, these genes of an individual to be tested can be screened for mutations which cause LQTS. Prediction of LQTS will enable practitioners to prevent this disorder using existing medical therapy.

SUMMARY OF THE INVENTION

The present invention relates to alterations in the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* and *KCNE2* genes and methods for detecting such alterations. The alterations in the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* and *KCNE2* genes include mutations and polymorphisms. Included among the mutations are frameshift, nonsense, splice, regulatory and missense mutations. Any method which is capable of detecting the alterations described herein can be used. Such methods include, but are not limited to, DNA sequencing, allele-specific probing, mismatch detection, single stranded conformation polymorphism detection and allele-specific PCR amplification.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a schematic representation of the predicted topology of KVLQT1 and the locations of LQTS-associated mutations. KVLQT1 consists of six putative transmembrane segments (S1 to S6) and a pore (Pore) region. Each circle represents an amino acid. The approximate location of LQTS-associated mutations identified in our laboratory are shown with filled circles.

Figure 2 is a schematic representation of HERG mutations. HERG consists of six putative transmembrane segments (S1 to S6) and a pore (Pore) region. Location of LQTS-associated mutations are shown with filled circles.

Figure 3 is a schematic representation of SCN5A and locations of LQTS-associated mutations. SCN5A consists of four domain (DI to DIV), each of which has six putative

transmembrane segments (S1 to S6) and a pore (Pore) region. Location of LQTS-associated mutations identified in our laboratory are shown with filled circles.

Figure 4 is a schematic representation of minK and locations of LQT-associated mutations. MinK consists of one putative transmembrane domain (S1). The approximate location of LQTS-associated mutations identified in our laboratory are shown with filled circles.

Figure 5 is a schematic representation of the predicted topology of MiRP1 and locations of arrhythmia-associated mutations. MiRP1 consists of one putative transmembrane domain (S1). The approximate location of arrhythmia-associated mutations identified in our laboratory are shown with filled circles.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to alterations in the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* and *KCNE2* genes and methods for detecting such alterations. The alterations in the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* and *KCNE2* genes include mutations and polymorphisms. Included among the mutations are frameshift, nonsense, splice, regulatory and missense mutations. Any method which is capable of detecting the mutations and polymorphisms described herein can be used. Such methods include, but are not limited to, DNA sequencing, allele-specific probing, mismatch detection, single stranded conformation polymorphism detection and allele-specific PCR amplification.

KVLQT1, *HERG*, *SCN5A*, *KCNE1* and *KCNE2* mutations cause increased risk for LQTS. Many different mutations occur in *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* and *KCNE2*. In order to detect the presence of alterations in the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* and *KCNE2* genes, a biological sample such as blood is prepared and analyzed for the presence or absence of a given alteration of *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2*. In order to detect the increased risk for LQTS or for the lack of such increased risk, a biological sample is prepared and analyzed for the presence or absence of a mutant allele of *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2*. Results of these tests and interpretive information are returned to the health care provider for communication to the tested individual. Such diagnoses may be performed by diagnostic

laboratories or, alternatively, diagnostic kits are manufactured and sold to health care providers or to private individuals for self-diagnosis.

The presence of hereditary LQTS may be ascertained by testing any tissue of a human for mutations of the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* gene. For example, a person who has inherited a germline *HERG* mutation would be prone to develop LQTS. This can be determined by testing DNA from any tissue of the person's body. Most simply, blood can be drawn and DNA extracted from the cells of the blood. In addition, prenatal diagnosis can be accomplished by testing fetal cells, placental cells or amniotic cells for mutations of the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* gene. Alteration of a wild-type *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* allele, whether, for example, by point mutation or deletion, can be detected by any of the means discussed herein.

There are several methods that can be used to detect DNA sequence variation. Direct DNA sequencing, either manual sequencing or automated fluorescent sequencing can detect sequence variation. Another approach is the single-stranded conformation polymorphism assay (SSCP) (Orita et al., 1989). This method does not detect all sequence changes, especially if the DNA fragment size is greater than 200 bp, but can be optimized to detect most DNA sequence variation. The reduced detection sensitivity is a disadvantage, but the increased throughput possible with SSCP makes it an attractive, viable alternative to direct sequencing for mutation detection on a research basis. The fragments which have shifted mobility on SSCP gels are then sequenced to determine the exact nature of the DNA sequence variation. Other approaches based on the detection of mismatches between the two complementary DNA strands include clamped denaturing gel electrophoresis (CDGE) (Sheffield et al., 1991), heteroduplex analysis (HA) (White et al., 1992) and chemical mismatch cleavage (CMC) (Grompe et al., 1989). None of the methods described above will detect large deletions, duplications or insertions, nor will they detect a regulatory mutation which affects transcription or translation of the protein. Other methods which might detect these classes of mutations such as a protein truncation assay or the asymmetric assay, detect only specific types of mutations and would not detect missense mutations. A review of currently available methods of detecting DNA sequence variation can be found in a recent review by Grompe (1993). Once a mutation is known, an allele specific detection approach such as allele specific oligonucleotide (ASO) hybridization can be utilized to rapidly screen large numbers of other samples for that same mutation. Such a technique can

utilize probes which are labeled with gold nanoparticles to yield a visual color result (Elghanian et al., 1997).

A rapid preliminary analysis to detect polymorphisms in DNA sequences can be performed by looking at a series of Southern blots of DNA cut with one or more restriction enzymes, preferably with a large number of restriction enzymes. Each blot contains a series of normal individuals and a series of LQTS cases. Southern blots displaying hybridizing fragments (differing in length from control DNA when probed with sequences near or including the *HERG* locus) indicate a possible mutation. If restriction enzymes which produce very large restriction fragments are used, then pulsed field gel electrophoresis (PFGE) is employed.

Detection of point mutations may be accomplished by molecular cloning of the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* alleles and sequencing the alleles using techniques well known in the art. Also, the gene or portions of the gene may be amplified, e.g., by PCR or other amplification technique, and the amplified gene or amplified portions of the gene may be sequenced.

There are six well known methods for a more complete, yet still indirect, test for confirming the presence of a susceptibility allele: 1) single stranded conformation analysis (SSCP) (Orita et al., 1989); 2) denaturing gradient gel electrophoresis (DGGE) (Wartell et al., 1990; Sheffield et al., 1989); 3) RNase protection assays (Finkelstein et al., 1990; Kinszler et al., 1991); 4) allele-specific oligonucleotides (ASOs) (Conner et al., 1983); 5) the use of proteins which recognize nucleotide mismatches, such as the *E. coli* mutS protein (Modrich, 1991); and 6) allele-specific PCR (Ruano and Kidd, 1989). For allele-specific PCR, primers are used which hybridize at their 3' ends to a particular *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* mutation. If the particular mutation is not present, an amplification product is not observed. Amplification Refractory Mutation System (ARMS) can also be used, as disclosed in European Patent Application Publication No. 0332435 and in Newton et al., 1989. Insertions and deletions of genes can also be detected by cloning, sequencing and amplification. In addition, restriction fragment length polymorphism (RFLP) probes for the gene or surrounding marker genes can be used to score alteration of an allele or an insertion in a polymorphic fragment. Such a method is particularly useful for screening relatives of an affected individual for the presence of the mutation found in that individual. Other techniques for detecting insertions and deletions as known in the art can be used.

In the first three methods (SSCP, DGGE and RNase protection assay), a new electrophoretic band appears. SSCP detects a band which migrates differentially because the sequence change causes a difference in single-strand, intramolecular base pairing. RNase protection involves cleavage of the mutant polynucleotide into two or more smaller fragments. DGGE detects differences in migration rates of mutant sequences compared to wild-type sequences, using a denaturing gradient gel. In an allele-specific oligonucleotide assay, an oligonucleotide is designed which detects a specific sequence, and the assay is performed by detecting the presence or absence of a hybridization signal. In the mutS assay, the protein binds only to sequences that contain a nucleotide mismatch in a heteroduplex between mutant and wild-type sequences.

Mismatches, according to the present invention, are hybridized nucleic acid duplexes in which the two strands are not 100% complementary. Lack of total homology may be due to deletions, insertions, inversions or substitutions. Mismatch detection can be used to detect point mutations in the gene or in its mRNA product. While these techniques are less sensitive than sequencing, they are simpler to perform on a large number of samples. An example of a mismatch cleavage technique is the RNase protection method. In the practice of the present invention, the method involves the use of a labeled riboprobe which is complementary to the human wild-type *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* gene coding sequence. The riboprobe and either mRNA or DNA isolated from the person are annealed (hybridized) together and subsequently digested with the enzyme RNase A which is able to detect some mismatches in a duplex RNA structure. If a mismatch is detected by RNase A, it cleaves at the site of the mismatch. Thus, when the annealed RNA preparation is separated on an electrophoretic gel matrix, if a mismatch has been detected and cleaved by RNase A, an RNA product will be seen which is smaller than the full length duplex RNA for the riboprobe and the mRNA or DNA. The riboprobe need not be the full length of the mRNA or gene but can be a segment of either. If the riboprobe comprises only a segment of the mRNA or gene, it will be desirable to use a number of these probes to screen the whole mRNA sequence for mismatches.

In similar fashion, DNA probes can be used to detect mismatches, through enzymatic or chemical cleavage. See, e.g., Cotton et al., 1988; Shenk et al., 1975; Novack et al., 1986. Alternatively, mismatches can be detected by shifts in the electrophoretic mobility of mismatched duplexes relative to matched duplexes. See, e.g., Cariello, 1988. With either riboprobes or DNA

probes, the cellular mRNA or DNA which might contain a mutation can be amplified using PCR (see below) before hybridization. Changes in DNA of the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* gene can also be detected using Southern hybridization, especially if the changes are gross rearrangements, such as deletions and insertions.

DNA sequences of the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* gene which have been amplified by use of PCR may also be screened using allele-specific probes. These probes are nucleic acid oligomers, each of which contains a region of the gene sequence harboring a known mutation. For example, one oligomer may be about 30 nucleotides in length, corresponding to a portion of the gene sequence. By use of a battery of such allele-specific probes, PCR amplification products can be screened to identify the presence of a previously identified mutation in the gene. Hybridization of allele-specific probes with amplified *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* sequences can be performed, for example, on a nylon filter. Hybridization to a particular probe under high stringency hybridization conditions indicates the presence of the same mutation in the tissue as in the allele-specific probe.

The newly developed technique of nucleic acid analysis via microchip technology is also applicable to the present invention. In this technique, literally thousands of distinct oligonucleotide probes are built up in an array on a silicon chip. Nucleic acid to be analyzed is fluorescently labeled and hybridized to the probes on the chip. It is also possible to study nucleic acid-protein interactions using these nucleic acid microchips. Using this technique one can determine the presence of mutations or even sequence the nucleic acid being analyzed or one can measure expression levels of a gene of interest. The method is one of parallel processing of many, even thousands, of probes at once and can tremendously increase the rate of analysis. Several papers have been published which use this technique. Some of these are Hacia et al., 1996; Shoemaker et al., 1996; Chee et al., 1996; Lockhart et al., 1996; DeRisi et al., 1996; Lipshutz et al., 1995. This method has already been used to screen people for mutations in the breast cancer gene *BRCA1* (Hacia et al., 1996). This new technology has been reviewed in a news article in Chemical and Engineering News (Borman, 1996) and been the subject of an editorial (Editorial, Nature Genetics, 1996). Also see Fodor (1997).

The most definitive test for mutations in a candidate locus is to directly compare genomic *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* sequences from patients with those from a control

population. Alternatively, one could sequence messenger RNA after amplification, e.g., by PCR, thereby eliminating the necessity of determining the exon structure of the candidate gene.

Mutations from patients falling outside the coding region of *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* can be detected by examining the non-coding regions, such as introns and regulatory sequences near or within the genes. An early indication that mutations in noncoding regions are important may come from Northern blot experiments that reveal messenger RNA molecules of abnormal size or abundance in patients as compared to control individuals.

Alteration of *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* mRNA expression can be detected by any techniques known in the art. These include Northern blot analysis, PCR amplification and RNase protection. Diminished mRNA expression indicates an alteration of the wild-type gene. Alteration of wild-type genes can also be detected by screening for alteration of wild-type KVLQT1, HERG, SCN5A, KCNE1 or KCNE2 protein. For example, monoclonal antibodies immunoreactive with HERG can be used to screen a tissue. Lack of cognate antigen would indicate a mutation. Antibodies specific for products of mutant alleles could also be used to detect mutant gene product. Such immunological assays can be done in any convenient formats known in the art. These include Western blots, immunohistochemical assays and ELISA assays. Any means for detecting an altered KVLQT1, HERG, SCN5A, KCNE1 or KCNE2 protein can be used to detect alteration of wild-type *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* genes. Functional assays, such as protein binding determinations, can be used. In addition, assays can be used which detect KVLQT1, HERG, SCN5A, KCNE1 or KCNE2 biochemical function. Finding a mutant *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* gene product indicates alteration of a wild-type *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* gene.

Mutant *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* genes or gene products can also be detected in other human body samples, such as serum, stool, urine and sputum. The same techniques discussed above for detection of mutant genes or gene products in tissues can be applied to other body samples. By screening such body samples, a simple early diagnosis can be achieved for hereditary LQTS.

Initially, the screening method involves amplification of the relevant *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* sequence. In another preferred embodiment of the invention, the screening method involves a non-PCR based strategy. Such screening methods include two-step label amplification methodologies that are well known in the art. Both PCR and non-PCR based

screening strategies can detect target sequences with a high level of sensitivity. Further details of these methods are briefly presented below and further descriptions can be found in PCT published application WO 96/05306, incorporated herein by reference.

The most popular method used today is target amplification. Here, the target nucleic acid sequence is amplified with polymerases. One particularly preferred method using polymerase-driven amplification is the polymerase chain reaction (PCR). The polymerase chain reaction and other polymerase-driven amplification assays can achieve over a million-fold increase in copy number through the use of polymerase-driven amplification cycles. Once amplified, the resulting nucleic acid can be sequenced or used as a substrate for DNA probes.

When the probes are used to detect the presence of the target sequences, the biological sample to be analyzed, such as blood or serum, may be treated, if desired, to extract the nucleic acids. The sample nucleic acid may be prepared in various ways to facilitate detection of the target sequence; e.g. denaturation, restriction digestion, electrophoresis or dot blotting. The targeted region of the analyte nucleic acid usually must be at least partially single-stranded to form hybrids with the targeting sequence of the probe. If the sequence is naturally single-stranded, denaturation will not be required. However, if the sequence is double-stranded, the sequence will probably need to be denatured. Denaturation can be carried out by various techniques known in the art.

Analyte nucleic acid and probe are incubated under conditions which promote stable hybrid formation of the target sequence in the probe with the putative targeted sequence in the analyte. The region of the probes which is used to bind to the analyte can be made completely complementary to the targeted region of the genes. Therefore, high stringency conditions are desirable in order to prevent false positives. However, conditions of high stringency are used only if the probes are complementary to regions of the chromosome which are unique in the genome. The stringency of hybridization is determined by a number of factors during hybridization and during the washing procedure, including temperature, ionic strength, base composition, probe length, and concentration of formamide. Under certain circumstances, the formation of higher order hybrids, such as triplexes, quadraplexes, etc., may be desired to provide the means of detecting target sequences.

Detection, if any, of the resulting hybrid is usually accomplished by the use of labeled probes. Alternatively, the probe may be unlabeled, but may be detectable by specific binding

with a ligand which is labeled, either directly or indirectly. Suitable labels, and methods for labeling probes and ligands are known in the art, and include, for example, radioactive labels which may be incorporated by known methods (e.g., nick translation, random priming or kinasing), biotin, fluorescent groups, chemiluminescent groups (e.g., dioxetanes, particularly triggered dioxetanes), enzymes, antibodies and the like. Variations of this basic scheme are known in the art, and include those variations that facilitate separation of the hybrids to be detected from extraneous materials and/or that amplify the signal from the labeled moiety. A number of these variations are well known.

As noted above, non-PCR based screening assays are also contemplated in this invention. This procedure hybridizes a nucleic acid probe (or an analog such as a methyl phosphonate backbone replacing the normal phosphodiester), to the low level DNA target. This probe may have an enzyme covalently linked to the probe, such that the covalent linkage does not interfere with the specificity of the hybridization. This enzyme-probe-conjugate-target nucleic acid complex can then be isolated away from the free probe enzyme conjugate and a substrate is added for enzyme detection. Enzymatic activity is observed as a change in color development or luminescent output resulting in a 10^3-10^6 increase in sensitivity. For example, the preparation of oligodeoxynucleotide-alkaline phosphatase conjugates and their use as hybridization probes are well known.

Two-step label amplification methodologies are known in the art. These assays work on the principle that a small ligand (such as digoxigenin, biotin, or the like) is attached to a nucleic acid probe capable of specifically binding the target gene. Allele specific probes are also contemplated within the scope of this example.

In one example, the small ligand attached to the nucleic acid probe is specifically recognized by an antibody-enzyme conjugate. In one embodiment of this example, digoxigenin is attached to the nucleic acid probe. Hybridization is detected by an antibody-alkaline phosphatase conjugate which turns over a chemiluminescent substrate. In a second example, the small ligand is recognized by a second ligand-enzyme conjugate that is capable of specifically complexing to the first ligand. A well known embodiment of this example is the biotin-avidin type of interactions. Methods for labeling nucleic acid probes and their use in biotin-avidin based assays are well known.

It is also contemplated within the scope of this invention that the nucleic acid probe assays of this invention will employ a cocktail of nucleic acid probes capable of detecting the gene or genes. Thus, in one example to detect the presence of *KVLQT1* in a cell sample, more than one probe complementary to *KVLQT1* is employed and in particular the number of different probes is alternatively 2, 3, or 5 different nucleic acid probe sequences. In another example, to detect the presence of mutations in the *KVLQT1* gene sequence in a patient, more than one probe complementary to *KVLQT1* is employed where the cocktail includes probes capable of binding to the allele-specific mutations identified in populations of patients with alterations in *KVLQT1*. In this embodiment, any number of probes can be used.

Large amounts of the polynucleotides of the present invention may be produced by replication in a suitable host cell. Natural or synthetic polynucleotide fragments coding for a desired fragment will be incorporated into recombinant polynucleotide constructs, usually DNA constructs, capable of introduction into and replication in a prokaryotic or eukaryotic cell. Usually the polynucleotide constructs will be suitable for replication in a unicellular host, such as yeast or bacteria, but may also be intended for introduction to (with and without integration within the genome) cultured mammalian or plant or other eukaryotic cell lines. The purification of nucleic acids produced by the methods of the present invention are described, e.g., in Sambrook et al., 1989 or Ausubel et al., 1992.

The polynucleotides of the present invention may also be produced by chemical synthesis, e.g., by the phosphoramidite method described by Beaucage and Caruthers (1981) or the triester method according to Matteucci and Caruthers (1981) and may be performed on commercial, automated oligonucleotide synthesizers. A double-stranded fragment may be obtained from the single-stranded product of chemical synthesis either by synthesizing the complementary strand and annealing the strand together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

Polynucleotide constructs prepared for introduction into a prokaryotic or eukaryotic host may comprise a replication system recognized by the host, including the intended polynucleotide fragment encoding the desired polypeptide, and will preferably also include transcription and translational initiation regulatory sequences operably linked to the polypeptide encoding segment. Expression vectors may include, for example, an origin of replication or autonomously replicating sequence (ARS) and expression control sequences, a promoter, an enhancer and

necessary processing information sites, such as ribosome-binding sites, RNA splice sites, polyadenylation sites, transcriptional terminator sequences, and mRNA stabilizing sequences. Such vectors may be prepared by means of standard recombinant techniques well known in the art and discussed, for example, in Sambrook et al. (1989) or Ausubel et al. (1992).

An appropriate promoter and other necessary vector sequences will be selected so as to be functional in the host, and may include, when appropriate, those naturally associated with the *KVLQT1* or other gene. Examples of workable combinations of cell lines and expression vectors are described in Sambrook et al. (1989) or Ausubel et al. (1992); see also, e.g., Metzger et al. (1988). Many useful vectors are known in the art and may be obtained from such vendors as Stratagene, New England Biolabs, Promega Biotech, and others. Promoters such as the trp, lac and phage promoters, tRNA promoters and glycolytic enzyme promoters may be used in prokaryotic hosts. Useful yeast promoters include promoter regions for metallothionein, 3-phosphoglycerate kinase or other glycolytic enzymes such as enolase or glyceraldehyde-3-phosphate dehydrogenase, enzymes responsible for maltose and galactose utilization, and others. Vectors and promoters suitable for use in yeast expression are further described in Hitzeman et al., EP 73,675A. Appropriate non-native mammalian promoters might include the early and late promoters from SV40 (Fiers et al., 1978) or promoters derived from murine Molony leukemia virus, mouse tumor virus, avian sarcoma viruses, adenovirus II, bovine papilloma virus or polyoma. Insect promoters may be derived from baculovirus. In addition, the construct may be joined to an amplifiable gene (e.g., DHFR) so that multiple copies of the gene may be made. For appropriate enhancer and other expression control sequences, see also Enhancers and Eukaryotic Gene Expression, Cold Spring Harbor Press, Cold Spring Harbor, New York (1983). See also, e.g., U.S. Patent Nos. 5,691,198; 5,735,500; 5,747,469 and 5,436,146.

While such expression vectors may replicate autonomously, they may also replicate by being inserted into the genome of the host cell, by methods well known in the art.

Expression and cloning vectors will likely contain a selectable marker, a gene encoding a protein necessary for survival or growth of a host cell transformed with the vector. The presence of this gene ensures growth of only those host cells which express the inserts. Typical selection genes encode proteins that a) confer resistance to antibiotics or other toxic substances, e.g. ampicillin, neomycin, methotrexate, etc., b) complement auxotrophic deficiencies, or c) supply critical nutrients not available from complex media, e.g., the gene encoding D-alanine

racemase for *Bacilli*. The choice of the proper selectable marker will depend on the host cell, and appropriate markers for different hosts are well known in the art.

The vectors containing the nucleic acids of interest can be transcribed *in vitro*, and the resulting RNA introduced into the host cell by well-known methods, e.g., by injection (see, Kubo et al. (1988)), or the vectors can be introduced directly into host cells by methods well known in the art, which vary depending on the type of cellular host, including electroporation; transfection employing calcium chloride, rubidium chloride calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; infection (where the vector is an infectious agent, such as a retroviral genome); and other methods. See generally, Sambrook et al. (1989) and Ausubel et al. (1992). The introduction of the polynucleotides into the host cell by any method known in the art, including, *inter alia*, those described above, will be referred to herein as "transformation." The cells into which have been introduced nucleic acids described above are meant to also include the progeny of such cells.

Large quantities of the nucleic acids and polypeptides of the present invention may be prepared by expressing the *KVLQT1* nucleic acid or portions thereof in vectors or other expression vehicles in compatible prokaryotic or eukaryotic host cells. The most commonly used prokaryotic hosts are strains of *Escherichia coli*, although other prokaryotes, such as *Bacillus subtilis* or *Pseudomonas* may also be used.

Mammalian or other eukaryotic host cells, such as those of yeast, filamentous fungi, plant, insect, or amphibian or avian species, may also be useful for production of the proteins of the present invention. Propagation of mammalian cells in culture is *per se* well known. See, Jakoby and Pastan (eds.) (1979). Examples of commonly used mammalian host cell lines are VERO and HeLa cells, Chinese hamster ovary (CHO) cells, and WI38, BHK, and COS cell lines, although it will be appreciated by the skilled practitioner that other cell lines may be appropriate, e.g., to provide higher expression, desirable glycosylation patterns, or other features. An example of a commonly used insect cell line is SF9.

Clones are selected by using markers depending on the mode of the vector construction. The marker may be on the same or a different DNA molecule, preferably the same DNA molecule. In prokaryotic hosts, the transformant may be selected, e.g., by resistance to ampicillin, tetracycline or other antibiotics. Production of a particular product based on temperature sensitivity may also serve as an appropriate marker.

Prokaryotic or eukaryotic cells transformed with the polynucleotides of the present invention will be useful not only for the production of the nucleic acids and polypeptides of the present invention, but also, for example, in studying the characteristics of KVLQT1 or other polypeptides.

The probes and primers based on the *KVLQT1* or other gene sequences disclosed herein are used to identify homologous *KVLQT1* or other gene sequences and proteins in other species. These gene sequences and proteins are used in the diagnostic/prognostic, therapeutic and drug screening methods described herein for the species from which they have been isolated.

The studies described in the Examples below resulted in the determination of many novel mutations. Previous studies had defined 126 distinct disease causing mutations in the LQTS genes *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* and *KCNE2* (Wang Q. et al., 1996a; Curran et al., 1995; Wang et al., 1995a; Splawski et al., 1997a; Abbott et al., 1999; Chouabe et al., 1997; Wollnik et al., 1997; Neyroud et al., 1997; Splawski et al., 1997b; Tyson et al., 1997; Schulze-Bahr et al., 1997; Priori et al., 1999; Splawski et al., 1998; Wang et al., 1995b; Russell et al., 1996; Neyroud et al., 1998; Neyroud et al., 1999; Donger et al., 1997; Tanaka et al., 1997; Jongbloed et al., 1999; Priori et al., 1998; Itoh et al., 1998a; Itoh et al., 1998b; Mohammad-Panah et al., 1999; Saarinen et al., 1998; Ackerman et al., 1998; Berthet et al., 1999; Kanters, 1998; van den Berg et al., 1997; Dausse et al., 1996; Benson et al., 1996; Akimoto et al., 1998; Satler et al., 1996; Satler et al., 1998; Makita et al., 1998, An et al., 1998; Schulze-Bahr et al., 1995; Duggal et al., 1998; Chen Q. et al., 1999; Li et al., 1998; Wei et al., 1999; Larsen et al., 1999a; Bianchi et al., 1999; Ackerman et al., 1999a; Ackerman et al., 1999b; Murray et al., 1999; Larsen et al., 1999b; Yoshida et al., 1999; Wattanasirichaigoon et al., 1999; Bezzina et al., 1999; Hoorntje et al., 1999). The sequence of each wild-type gene has been published. The *KVLQT1* can be found in Splawski et al. (1998) and the coding region of the cDNA is shown herein as SEQ ID NO:1 and the encoded KVLQT1 is shown as SEQ ID NO:2. SCN5A was reported by Gellens et al. (1992) and its sequence is provided by GenBank Accession No. NM_000335. The coding sequence of *SCN5A* is shown herein as SEQ ID NO:3 and the encoded SCN5A is shown as SEQ ID NO:4. Most of the mutations were found in *KVLQT1* (Yoshida et al., 1999) and *HERG* (Itoh et al., 1998b), and fewer in *SCN5A* (Wang Q. et al., 1996a), *KCNE1* (Jiang et al., 1994) and *KCNE2* (Ward, 1964). These mutations were identified in regions with known intron/exon structure, primarily the transmembrane and pore domains. In this study, we screened 262 individuals with

LQTS for mutations in all known arrhythmia genes. We identified 134 mutations, 80 of which were novel. Together with 43 mutations reported in our previous studies, we have now identified 177 mutations in these 262 LQTS individuals (68%). The failure to identify mutations in 32% of the individuals may result from phenotypic errors, incomplete sensitivity of SSCP or presence of mutations in regulatory sequences. However, it is also clear that additional LQTS genes await discovery (Jiang et al., 1994; Schott et al., 1995).

Missense mutations were most common (72%), followed by frameshift mutations (10%), in-frame deletions, nonsense and splice site mutations (5-7% each). Most mutations resided in intracellular (52%) and transmembrane (30%) domains; 12% were found in pore and 6% in extracellular segments. One hundred one of the 129 distinct LQTS mutations (78%) were identified in single families or individuals. Most of the 177 mutations were found in *KVLQT1* (75 or 42%) and *HERG* (80 or 45%). These two genes accounted for 87% of the identified mutations, while mutations in *SCN5A* (14 or 8%), *KCNE1* (5 or 3%) and *KCNE2* (3 or 2%) accounted for the other 13%.

Multiple mutations were found in regions encoding S5, S5/P, P and S6 of *KVLQT1* and *HERG*. The P region of potassium channels forms the outer pore and contains the selectivity filter (Doyle et al., 1998). Transmembrane segment 6, corresponding to the inner helix of KcsA, forms the inner 2/3 of the pore. This structure is supported by the S5 transmembrane segment, corresponding to the outer helix of KcsA, and is conserved from prokaryotes to eukaryotes ((MacKinnon et al., 1998). Mutations in these regions will likely disrupt potassium transport. Many mutations were identified in the C-termini of *KVLQT1* and *HERG*. Changes in the C-terminus of HERG could lead to anomalies in tetramerization as it has been proposed that the C-terminus of eag, which is related to HERG, is involved in this process (Ludwig et al, 1994).

Multiple mutations were also identified in regions that were different for *KVLQT1* and *HERG*. In *KVLQT1*, multiple mutations were found in the sequences coding for the S2/S3 and S4/S5 linkers. Coexpression of S2/S3 mutants with wild-type KVLQT1 in *Xenopus* oocytes led to simple loss of function or dominant-negative effect without significantly changing the biophysical properties of I_{Ks} channels (Chouabe et al., 1997; Shalaby et al., 1997; Wang et al., 1999). On the other hand, S4/S5 mutations altered the gating properties of the channels and modified KVLQT1 interactions with minK subunits (Wang et al., 1999; Franqueza et al., 1999).

In *HERG*, more than 20 mutations were identified in the N-terminus. HERG channels lacking this region deactivate faster and mutations in the region had a similar effect (Chen J. et al., 1999).

Mutations in *KCNE1* and *KCNE2*, encoding minK and MiRP1, the respective I_{Ks} and I_{Kr} β-subunits, altered the biophysical properties of the channels (Splawski et al., 1997a; Abbott et al., 1999; Sesti and Goldstein, 1998). A MiRP1 mutant, involved in clarithromyocin-induced arrhythmia, increased channel blockade by the antibiotic (Abbott et al., 1999). Mutations in *SCN5A*, the sodium channel α-subunit responsible for cardiac I_{Na}, destabilized the inactivation gate causing delayed channel inactivation and dispersed reopenings (Bennett et al., 1995; Dumaine et al., 1996; Wei et al., 1999; Wang DW et al., 1996). One SCN5A mutant affected the interactions with the sodium channel β-subunit (An et al., 1998).

It is interesting to note that probands with *KCNE1* and *KCNE2* mutations were older and had shorter QTc than probands with the other genotypes. The significance of these differences is unknown, however, as the number of probands with *KCNE1* and *KCNE2* genotypes was small.

This catalogue of mutations will facilitate genotype-phenotype analyses. It also has clinical implications for presymptomatic diagnosis and, in some cases, for therapy. Patients with mutations in *KVLQT1*, *HERG*, *KCNE1* and *KCNE2*, for example, may benefit from potassium therapy (Compton et al., 1996). Sodium channel blockers, on the other hand, might be helpful in patients with *SCN5A* mutations (Schwartz et al. (1995). The identification of mutations is of importance for ion channel studies as well. The expression of mutant channels in heterologous systems can reveal how structural changes influence the behavior of the channel or how mutations affect processing (Zhou et al., 1998; Furutani et al., 1999). These studies improve our understanding of channel function and provide insights into mechanisms of disease. Finally, mutation identification will contribute to the development of genetic screening for arrhythmia susceptibility.

The present invention is described by reference to the following Examples, which are offered by way of illustration and are not intended to limit the invention in any manner. Standard techniques well known in the art or the techniques specifically described in the Examples were utilized.

Example 1

Ascertainment and Phenotyping

Individuals were ascertained in clinics from North America and Europe. Individuals were evaluated for LQTS based on QTc (the QT interval corrected for heart rate) and for the presence of symptoms. In this study, we focused on the probands. Individuals show prolongation of the QT interval (QTc≥460 ms) and/or documented *torsade de pointes*, ventricular fibrillation, cardiac arrest or aborted sudden death. Informed consent was obtained in accordance with local institutional review board guidelines. Phenotypic data were interpreted without knowledge of genotype. Sequence changes altering coding regions or predicted to affect splicing that were not detected in at least 400 control chromosomes were defined as mutations. No changes except known polymorphisms were detected ina ny of the genes in the control population. This does not exclude the possibility that some mutations are rare variants not associated with disease.

Example 2

Mutational Analyses

To determine the spectrum of LQTS mutations, we used SSCP (Single Stand Conformation Polymorphism) and DNA sequence analyses to screen 262 unrelated individuals with LQTS. Seventeen primer pairs were used to screen *KVLQT1* (Splawski et al., 1998), twenty-one primer pairs were used for *HERG* (Splawski et al., 1998) and three primer pairs were used for *KCNE1* (Splawski et al., 1997a) and *KCNE2* (Abbott et al., 1999). Thirty-three primer pairs (Wang Q. et al., 1996b) were used in SSCP analysis to screen all *SCN5A* exons in 50 individuals with suspected abnormalities in I_{Na}. Exons 23-28, in which mutations were previously identified, were screened in all 262 individuals.

Gender, age, QTc and presence of symptoms are summarized in Table 1. The average age at ascertainment was 29 with a corrected QT interval of 492 ms. Seventy-five percent had a history of symptoms and females predominated with an ~ 2:1 ratio. Although the numbers were small, corrected QT intervals for individuals harboring *KCNE1* and *KCNE2* mutations were shorter at 457 ms.

Table 1

Age, QTc, Gender and Presence of Symptoms

Genotype	Age*, y (mean±SD)	Gender (F/M)	QTc, ms (mean±SD)	Symptoms†
KVLQT1	32 ± 19	52/23	493 ± 45	78%
HERG	31 ± 19	51/29	498 ± 48	71%
SCN5A	32 ± 24	8/6	511 ± 42	55%
KCNE1	43 ± 16	3/2	457 ± 25	40%
KCNE2	54 ± 20	3/0	457 ± 05	67%
unknown	25 ± 16	56/29	484 ± 46	81%
all	29 ± 19	173/89	492 ± 47	75%

* - age at ascertainment

† - symptoms include syncope, cardiac arrest or sudden death

The SSCP analyses revealed many mutations. *KVLQT1* mutations associated with LQTS were identified in 52 individuals (Figure 1 and Table 2). Twenty of the mutations were novel. *HERG* mutations were identified in 68 LQTS individuals (Figure 2 and Table 3). Fifty-two of these mutations were novel. *SCN5A* mutations were identified in eight cases (Figure 3 and Table 4). Five of the mutations were novel. Three novel *KCNE1* mutations were identified (Figure 4 and Table 5) and three mutations were identified in *KCNE2* (Figure 5 and Table 6) (Abbott et al., 1999). None of the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* and *KCNE2* mutations was observed in 400 control chromosomes.

Table 2

Summary of All *KVLQT1* Mutations*

Nucleotide Change†	Coding Effect	Position	Exon	Number of families‡	Study
del211-219	del71-73	N-terminus	1	1	Ackerman et al., 1999a
A332G †	Y111C	N-terminus	1	1	This

Nucleotide Change†	Coding Effect	Position	Exon	Number of families‡	Study
del451-452	A150fs/132	S2	2	1 JLN	Chen Q. et al., 1999
T470G	F157C	S2	1	1	Larsen et al., 1999a
G477+1A	M159sp	S2	2	1 JLN, 1 UK	This; Donger et al., 1997
G477+5A	M159sp	S2	1	1	Ackerman et al., 1999b
G478A †	E160K	S2	3	1	This
del500-502	F167W/del G168	S2	3	1	Wang Q. et al., 1996a
G502A	G168R	S2	3	7	This; Splawski et al., 1998; Donger et al., 1997
C520T	R174C	S2/S3	3	1	Donger et al., 1997
G521A †	R174H	S2/S3	3	1	This
G532A	A178T	S2/S3	3	1	Tanaka et al., 1997
G532C	A178P	S2/S3	3	1	Wang Q. et al., 1996a
G535A †	G179S	S2/S3	3	1	This
A551C	Y184S	S2/S3	3	2	This; Jongbloed et al., 1999
G565A	G189R	S2/S3	3	3	Wang Q. et al., 1996a; Jongbloed et al., 1999
insG567-568	G189fs/94	S2/S3	3	1 (RW + JLN)	Splawski et al., 1997b
G569A	R190Q	S2/S3	3	2	Splawski et al., 1998; Donger et al., 1997
del572-576	L191fs/90	S2/S3	3	1 JLN, 1 RW 2 (JLN + RW)	Tyson et al., 1997; Ackerman et al., 1999b

Nucleotide Change†	Coding Effect	Position	Exon	Number of families‡	Study
G580C †	A194P	S2/S3	3	1	This
C674T	S225L	S4	4	2	This; Priori et al., 1999
G724A	D242N	S4/S5	5	1	Itoh et al., 1998b
C727T †	R243C	S4/S5	5	2	This
G728A	R243H	S4/S5	5	1 JLN	Saarinen et al., 1998
T742C †	W248R	S4/S5	5	1	This
T749A	L250H	S4/S5	5	1	Itoh et al., 1998a
G760A	V254M	S4/S5	5	4	This; Wang Q. et al., 1996a; Donger et al., 1997
G781A	E261K	S4/S5	6	1	Donger et al., 1997
T797C †	L266P	S5	6	1	This
G805A	G269S	S5	6	1	Ackerman et al., 1999b
G806A	G269D	S5	6	3	This; Donger et al., 1997
C817T	L273F	S5	6	2	This; Wang Q. et al., 1996a
A842G	Y281C	S5	6	1	Priori et al., 1999
G898A	A300T	S5/Pore	6	1	Priori et al., 1998
G914C	W305S	Pore	6	1 JLN	Chouabe et al., 1997
G916A	G306R	Pore	6	1	Wang Q. et al, 1996a
del921-(921+2)	V307sp	Pore	6	1	Li et al., 1998
G921+1T †	V307sp	Pore	6	1	This
A922-2C †	V307sp	Pore	7	1	This
G922-1C	V307sp	Pore	7	1	Murray et al., 1999
C926G	T309R	Pore	7	1	Donger et al., 1997

Nucleotide Change†	Coding Effect	Position	Exon	Number of families‡	Study
G928A †	V310I	Pore	7	1	This
C932T	T311I	Pore	7	1	Saarinen et al., 1998
C935T	T312I	Pore	7	2	This; Wang Q. et al., 1996a
C939G	I313M	Pore	7	1	Tanaka et al., 1997
G940A	G314S	Pore	7	7	Splawski et al., 1998; Russell et al., 1996; Donger et al., 1997; Jongbloed et al., 1999; Itoh et al., 1998b
A944C	Y315S	Pore	7	3	Donger et al., 1997; Jongbloed et al., 1999
A944G	Y315C	Pore	7	2	Priori et al., 1999; Splawski et al., 1998
G949A	D317N	Pore	7	2	Wollnik et al., 1997; Saarinen et al., 1998
G954C	K318N	Pore	7	1	Splawski et al., 1998
C958G	P320A	Pore	7	1	Donger et al., 1997
G973A	G325R	S6	7	4	This; Donger et al., 1997; Tanaka et al., 1997
del1017-1019	delF340	S6	7	2	This; Ackerman et al., 1998
C1022A	A341E	S6	7	5	This; Wang Q. et al., 1996a; Berthet et al., 1999

Nucleotide Change†	Coding Effect	Position	Exon	Number of families‡	Study
C1022T	A341V	S6	7	7	This; Wang Q. et al., 1996a; Russell et al., 1996; Donger et al., 1997; Li et al., 1998
C1024T	L342F	S6	7	1	Donger et al., 1997
C1031T	A344V	S6	7	1	Donger et al., 1997
G1032A	A344sp	S6	7	9	This; Kanters, 1998; Li et al., 1998; Ackerman et al., 1999b; Murray et al., 1999
G1032C	A344sp	S6	7	1	Murray et al., 1999
G1033C	G345R	S6	8	1	van den Berg et al., 1997
G1034A	G345E	S6	8	1	Wang Q. et al., 1996a
C1046G †	S349W	S6	8	1	This
T1058C	L353P	S6	8	1	Splawski et al., 1998
C1066T †	Q356X	C-terminus	8	1	This
C1096T	R366W	C-terminus	8	1	Splawski et al., 1998
G1097A †	R366Q	C-terminus	8	1	This
G1097C	R366P	C-terminus	8	1	Tanaka et al., 1997
G1111A	A371T	C-terminus	8	1	Donger et al., 1997
T1117C	S373P	C-terminus	8	1	Jongbloed et al., 1999
C1172T †	T391I	C-terminus	9	1	This
T1174C	W392R	C-terminus	9	1	Jongbloed et al., 1999
C1343G †	P448R	C-terminus	10	2	This
C1522T	R518X	C-terminus	12	1 JLN, 3 RW	This; Larsen et al., 1999

Nucleotide Change[†]	Coding Effect	Position	Exon	Number of families[‡]	Study
G1573A	A525T	C-terminus	12	1	Larsen et al., 1999b
C1588T †	Q530X	C-terminus	12	1 JLN, 1 RW	This
C1615T	R539W	C-terminus	13	1	Chouabe et al., 1997
del6/ins7	E543fs/107	C-terminus	13	1 JLN	Neyroud et al., 1997
C1663T	R555C	C-terminus	13	3	Donger et al., 1997
C1697T †	S566F	C-terminus	14	3	This
C1747T †	R583C	C-terminus	15	1	This
C1760T	T587M	C-terminus	15	1 JLN, 1 RW	Donger et al., 1997; Itoh et al., 1998b
G1772A	R591H	C-terminus	15	1	Donger et al., 1997
G1781A †	R594Q	C-terminus	15	3	This
del1892-1911	P630fs/13	C-terminus	16	1 JLN	Donger et al., 1997
insC1893-1894	P631fs/19	C-terminus	16	1	Donger et al., 1997

[*] - ins denotes insertion; del denotes deletion; sp denotes the last unaffected amino acid before the predicted splice mutation; fs denotes the last amino acid unaffected by a frameshift, following fs is the number of amino acids before termination; X denotes a stop codon occurred.

† - denotes novel mutation

‡ - Number of Romano-Ward families unless otherwise indicated (UK - unknown)

Table 3

Summary of All *HERG* Mutations*

Nucleotide Change	Coding Effect	Position	Exon	Number of RW Families	Study
C87A †	F29L	N-terminus	2	1	This
A98C †	N33T	N-terminus	2	2	This
C132A †	C44X	N-terminus	2	1	This
G140T †	G47V	N-terminus	2	1	This
G157C †	G53R	N-terminus	2	1	This
G167A †	R56Q	N-terminus	2	1	This
T196G †	C66G	N-terminus	2	1	This
A209G †	H70R	N-terminus	2	2	This
C215A †	P72Q	N-terminus	2	2	This
del221-251 †	R73fs/31	N-terminus	2	1	This
G232C †	A78P	N-terminus	2	1	This
dupl234-250†	A83fs/37	N-terminus	2	1	This
C241T †	Q81X	N-terminus	2	1	This
T257G †	L86R	N-terminus	2	1	This
insC422-423†	P141fs/2	N-terminus	3	1	This
insC453-454†	P151fs/179	N-terminus	3	1	This
dupl558-600	L200fs/144	N-terminus	4	1	Hoorntje et al., 1999
insC724-725†	P241fs/89	N-terminus	4	1	This
del885 †	V295fs/63	N-terminus	4	1	This
C934T †	R312C	N-terminus	5	1	This
C1039T †	P347S	N-terminus	5	1	This
G1128A †	Q376sp	N-terminus	5	1	This

Nucleotide Change	Coding Effect	Position	Exon	Number of RW Families	Study
A1129-2G †	Q376sp	N-terminus	6	1	This
del1261	Y420fs/12	S1	6	1	Curran et al., 1995
C1283A	S428X	S1/S2	6	1	Priori et al., 1999
C1307T	T436M	S1/S2	6	1	Priori et al., 1999
A1408G	N470D	S2	6	1	Curran et al., 1995
C1421T	T474I	S2/S3	6	1	Tanaka et al., 1997
C1479G	Y493X	S2/S3	6	1	Itoh et al., 1998a
del1498-1524	del500-508	S3	6	1	Curran et al., 1995
G1592A †	R531Q	S4	7	1	This
C1600T	R534C	S4	7	1	Itoh et al., 1998a
T1655C †	L552S	S5	7	1	This
delT1671	T556fs/7	S5	7	1	Schulze-Bahr et al., 1995
G1672C	A558P	S5	7	1	Jongbloed et al., 1999
G1681A	A561T	S5	7	4	This; Dausse et al., 1996
C1682T	A561V	S5	7	4	This; Curran et al., 1995; Priori et al., 1999
G1714C	G572R	S5/Pore	7	1	Larsen et al., 1999a
G1714T	G572C	S5/Pore	7	1	Splawski et al., 1998
C1744T	R582C	S5/Pore	7	1	Jongbloed et al., 1999
G1750A †	G584S	S5/Pore	7	1	This
G1755T †	W585C	S5/Pore	7	1	This
A1762G	N588D	S5/Pore	7	1	Splawski et al., 1998
T1778C †	I593T	S5/Pore	7	1	This
T1778G	I593R	S5/Pore	7	1	Benson et al., 1996
G1801A	G601S	S5/Pore	7	1	Akimoto et al., 1998

Nucleotide Change	Coding Effect	Position	Exon	Number of RW Families	Study
G1810A	G604S	S5/Pore	7	2	This; Jongbloed et al., 1999
G1825A †	D609N	S5/Pore	7	1	This
T1831C	Y611H	S5/Pore	7	1	Tanaka et al., 1997
T1833 (A or G)	Y611X	S5/Pore	7	1	Schulze-Bahr et al., 1995
G1834T	V612L	Pore	7	1	Satler et al., 1998
C1838T	T613M	Pore	7	4	This; Jongbloed et al., 1999
C1841T	A614V	Pore	7	6	Priori et al., 1999; Splawski et al., 1998; Tanaka et al., 1997; Satler et al., 1998
C1843G †	L615V	Pore	7	1	This
G1876A †	G626S	Pore	7	1	This
C1881G †	F627L	Pore	7	1	This
G1882A	G628S	Pore	7	2	This; Curran et al., 1995
A1885G	N629D	Pore	7	1	Satler et al., 1998
A1886G	N629S	Pore	7	1	Satler et al., 1998
C1887A	N629K	Pore	7	1	Yoshida et al., 1999
G1888C	V630L	Pore	7	1	Tanaka et al., 1997
T1889C	V630A	Pore	7	1	Splawski et al., 1998
C1894T †	P632S	Pore	7	1	This
A1898G	N633S	Pore	7	1	Satler et al., 1998
A1912G †	K638E	S6	7	1	This
del1913-1915†	delK638	S6	7	1	This

Nucleotide Change	Coding Effect	Position	Exon	Number of RW Families	Study
C1920A	F640L	S6	7	1	Jongbloed et al., 1999
A1933T †	M645L	S6	7	1	This
del1951-1952	L650fs/2	S6	8	1	Itoh et al., 1998a
G2044T †	E682X	S6/cNBD	8	1	This
C2173T	Q725X	S6/cNBD	9	1	Itoh et al., 1998a
insT2218-2219 †	H739fs/63	S6/cNBD	9	1	This
C2254T †	R752W	S6/cNBD	9	1	This
dupl2356-2386	V796fs/22	cNBD	9	1	Itoh et al., 1998a
del2395 †	I798fs/10	cNBD	9	1	This
G2398+1C	L799sp	cNBD	9	2	This; Curran et al., 1995
T2414C †	F805S	cNBD	10	1	This
T2414G †	F805C	cNBD	10	1	This
C2453T	S818L	cNBD	10	1	Berthet et al., 1999
G2464A	V822M	cNBD	10	2	Berthet et al., 1999; Satler et al., 1996
C2467T †	R823W	cNBD	10	2	This
A2582T †	N861I	C-terminus	10	1	This
G2592+1A	D864sp	C-terminus	10	2	This; Berthet et al., 1999
del2660 †	K886fs/85	C-terminus	11	1	This
C2750T †	P917L	C-terminus	12	1	This
del2762 †	R920fs/51	C-terminus	12	1	This
C2764T †	R922W	C-terminus	12	1	This
insG2775-2776 †	G925fs/13	C-terminus	12	1	This
del2906 †	P968fs/4	C-terminus	12	1	This

Nucleotide Change	Coding Effect	Position	Exon	Number of RW Families	Study
del2959-2960†	P986fs/130	C-terminus	12	1	This
C3040T †	R1014X	C-terminus	13	2	This
del3094 †	G1031fs/24	C-terminus	13	1	This
insG3107-3108	G1036fs/82	C-terminus	13	1	Berthet et al., 1999
insC3303-3304 †	P1101fs	C-terminus	14	1	This

* - all characters same as in Table 2

Table 4

Summary of All *SCN5A* Mutations

Nucleotide Change	Coding Effect	Position	Exon	Number of RW Families	Study
G3340A †	D1114N	DII/DIII	18	1	This
C3911T	T1304M	DIII/S4	22	1	Wattanasirichaigoon et al., 1999
A3974G	N1325S	DIII/S4/S5	23	1	Wang et al., 1995b
C4501G †	L1501V	DIII/DIV	26	1	This
del4511-4519	del1505 - 1507	DIII/DIV	26	4	Wang et al., 1995a; Wang et al., 1995b
del4850-4852 †	delF1617	DIV/S3/S4	28	1	This
G4868A	R1623Q	DIV/S4	28	2	This; Makita et al., 1998
G4868T †	R1623L	DIV/S4	28	1	This

Nucleotide Change	Coding Effect	Position	Exon	Number of RW Families	Study
G4931A	R1644H	DIV/S4	28	2	This; Wang et al., 1995b
C4934T	T1645M	DIV/S4	28	1	Wattanasirichaigoon et al., 1999
G5350A †	E1784K	C-terminus	28	2	This; Wei et al., 1999
G5360A †	S1787N	C-terminus	28	1	This
A5369G	D1790G	C-terminus	28	1	An et al., 1998
insTGA 5385-5386	insD1795 -1796	C-terminus	28	1	Bezzina et al., 1999

* - all characters same as in Table 2. Fifty individuals with suspected abnormalities in I_{Na} were screened for all *SCN5A* exons. All individuals were screened for exons 23-28.

Table 5

Summary of All *KCNE1* Mutations*

Nucleotide Change	Coding Effect	Position	Exon	Number of Families	Study
C20T	T7I	N-terminus	3	1 JLN	Schulze-Bahr et al., 1997
G95A †	R32H	N-terminus	3	1	This
G139T	V47F	S1	3	1 JLN	Bianchi et al., 1999
TG151-152AT	L51H	S1	3	1 JLN	Bianchi et al., 1999
A172C/TG176-177CT	TL58-59PP	S1	3	1 JLN	Tyson et al., 1997
C221T	S74L	C-terminus	3	1	Splawski et al., 1997a

Nucleotide Change	Coding Effect	Position	Exon	Number of Families	Study
G226A	D76N	C-terminus	3	1 JLN, 1 RW, 1 (JLN + RW)	Splawski et al., 1997a; Tyson et al., 1997; Duggal et al., 1998
T259C	W87R	C-terminus	3	1	Bianchi et al., 1999
C292T †	R98W	C-terminus	3	1	This
C379A †	P127T	C-terminus	3	1	This

* - all characters same as in Table 2

Table 6

Summary of All *KCNE2* Mutations

Nucleotide Change	Coding Effect	Position	Exon	Number of Families	Study
C25G	Q9E	N-terminus	1	1	Abbott et al., 1999
T161T	M54T	S1	1	1	Abbott et al., 1999
T170C	I57T	S1	1	1	Abbott et al., 1999

Table 7

Mutations by Type

Type	*KVLQT1*	*HERG*	*SCN5A*	*KCNE1*	*KCNE2*	Total
Missense	59	52	9	5	3	128
Nonsense	6	5	0	0	0	11
AA deletion*	2	2	5	0	0	9
Frameshift	1	16	0	0	0	17
Splice	7	5	0	0	0	12
Total	75	80	14	5	3	177

* - AA denotes amino acid

Table 8

Mutations by Position

Gene Protein Position	*KVLQT1* KVLQT1	*HERG* HERG	*SCN5A* SCN5A	*KCNE1* minK	*KCNE2* MiRP1	Total
Extracellular	0	7	1	1	1	10
Transmembrane	33	13	5	0	2	53
Pore	9	12	0	N/A	N/A	21
Intracellular	33	48	8	4	0	93
Total	75	80	14	5	3	177

While the invention has been disclosed in this patent application by reference to the details of preferred embodiments of the invention, it is to be understood that the disclosure is intended in an illustrative rather than in a limiting sense, as it is contemplated that modifications will readily occur to those skilled in the art, within the spirit of the invention and the scope of the appended claims.

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WHAT IS CLAIMED IS:

1. An isolated DNA comprising a sequence of SEQ ID NO:1 as altered by one or more mutations selected from the group consisting of A332G, G478A, G521A, G535A, G580C, C727T, T742C, T797C, G921+1T, A922-2C, G928A, C1046G, C1066T, G1097A, C1172T, C1343G, C1588T, C1697T, C1747T and G1781A.

2. A nucleic acid probe specifically hybridizable to a human mutated *KVLQT1* and not to wild-type DNA, said mutated *KVLQT1* comprising a mutation of SEQ ID NO:1 selected from the group consisting of A332G, G478A, G521A, G535A, G580C, C727T, T742C, T797C, G921+1T, A922-2C, G928A, C1046G, C1066T, G1097A, C1172T, C1343G, C1588T, C1697T, C1747T and G1781A.

3. A method for detecting a mutation in *KVLQT1* said mutation selected from the group consisting of A332G, G478A, G521A, G535A, G580C, C727T, T742C, T797C, G921+1T, A922-2C, G928A, C1046G, C1066T, G1097A, C1172T, C1343G, C1588T, C1697T, C1747T and G1781A which comprises analyzing a sequence of said gene or RNA from a human sample or analyzing the sequence of cDNA made from mRNA from said sample.

4. The method of claim 3 wherein said mutation is detected by a method selected from the group consisting of:

a) hybridizing a probe specific for one of said mutations to RNA isolated from said human sample and detecting the presence of a hybridization product, wherein the presence of said product indicates the presence of said mutation in the sample;

b) hybridizing a probe specific for one of said mutations to cDNA made from RNA isolated from said sample and detecting the presence of a hybridization product, wherein the presence of said product indicates the presence of said mutation in the sample;

c) hybridizing a probe specific for one of said mutations to genomic DNA isolated from said sample and detecting the presence of a hybridization product, wherein the presence of said product indicates the presence of said mutation in the sample;

d) amplifying all or part of said gene in said sample using a set of primers to produce amplified nucleic acids and sequencing the amplified nucleic acids;

e) amplifying part of said gene in said sample using a primer specific for one of said mutations and detecting the presence of an amplified product, wherein the presence of said product indicates the presence of said mutation in the sample;

f) molecularly cloning all or part of said gene in said sample to produce a cloned nucleic acid and sequencing the cloned nucleic acid;

g) amplifying said gene to produce amplified nucleic acids, hybridizing the amplified nucleic acids to a DNA probe specific for one of said mutations and detecting the presence of a hybridization product, wherein the presence of said product indicates the presence of said mutation;

h) forming single-stranded DNA from a gene fragment of said gene from said human sample and single-stranded DNA from a corresponding fragment of a wild-type gene, electrophoresing said single-stranded DNAs on a non-denaturing polyacrylamide gel and comparing the mobility of said single-stranded DNAs on said gel to determine if said single-stranded DNA from said sample is shifted relative to wild-type and sequencing said single-stranded DNA having a shift in mobility;

i) forming a heteroduplex consisting of a first strand of nucleic acid selected from the group consisting of a genomic DNA fragment isolated from said sample, an RNA fragment isolated from said sample and a cDNA fragment made from mRNA from said sample and a second strand of a nucleic acid consisting of a corresponding human wild-type gene fragment, analyzing for the presence of a mismatch in said heteroduplex, and sequencing said first strand of nucleic acid having a mismatch;

j) forming single-stranded DNA from said gene of said human sample and from a corresponding fragment of an allele specific for one of said mutations, electrophoresing said single-stranded DNAs on a non-denaturing polyacrylamide gel and comparing the mobility of said single-stranded DNAs on said gel to determine if said single-stranded DNA from said sample is shifted relative to said allele, wherein no shift in electrophoretic mobility of the single-stranded DNA relative to the allele indicates the presence of said mutation in said sample; and

k) forming a heteroduplex consisting of a first strand of nucleic acid selected from the group consisting of a genomic DNA fragment of said gene isolated from said sample, an RNA fragment isolated from said sample and a cDNA fragment made from mRNA from said sample and a second strand of a nucleic acid consisting of a corresponding gene allele fragment specific for one of said mutations and analyzing for the presence of a mismatch in said heteroduplex, wherein no mismatch indicates the presence of said mutation.

5. A method according to claim 4 wherein hybridization is performed *in situ*.

6. An isolated human polypeptide encoded by *KVLQT1* comprising a mutation of SEQ ID NO:2 selected from the group consisting of Y111C, E160K, R174H, G179S, A194P, R243C, W248R, L266P, V307sp, V310I, S349W, Q356X, R366Q, T391I, P448R, Q530X, S566F, R583C and R594Q.

7. An antibody capable of binding the polypeptide of claim 6 but incapable of binding a wild-type polypeptide.

8. An antibody according to claim 7 wherein said antibody is a monoclonal antibody.

9. A method of assessing a risk in a human subject for long QT syndrome which comprises screening said subject for a mutation in *KVLQT1* by comparing the sequence of said *KVLQT1* or its expression products isolated from a tissue sample of said subject with a wild-type sequence of said *KVLQT1* or its expression products, wherein a mutation in the sequence of the subject indicates a risk for long QT syndrome.

10. The method of claim 9 wherein said expression product is selected from mRNA of said gene or a polypeptide encoded by said gene.

11. The method of claim 9 wherein one or more of the following procedures is carried out:

(a) observing shifts in electrophoretic mobility of single-stranded DNA from said sample on non-denaturing polyacrylamide gels;

(b) hybridizing a probe to genomic DNA isolated from said sample under conditions suitable for hybridization of said probe to said gene;

(c) determining hybridization of an allele-specific probe to genomic DNA from said sample;

(d) amplifying all or part of said gene from said sample to produce an amplified sequence and sequencing the amplified sequence;

(e) determining by nucleic acid amplification the presence of a specific mutant allele in said sample;

(f) molecularly cloning all or part of said gene from said sample to produce a cloned sequence and sequencing the cloned sequence;

(g) determining whether there is a mismatch between molecules (1) said gene genomic DNA or mRNA isolated from said sample, and (2) a nucleic acid probe complementary to the human wild-type gene DNA, when molecules (1) and (2) are hybridized to each other to form a duplex;

(h) amplification of said gene sequences in said sample and hybridization of the amplified sequences to nucleic acid probes which comprise wild-type gene sequences;

(i) amplification of said gene sequences in said tissue and hybridization of the amplified sequences to nucleic acid probes which comprise said mutant gene sequences;

(j) screening for a deletion mutation;

(k) screening for a point mutation;

(l) screening for an insertion mutation;

(m) determining *in situ* hybridization of said gene in said sample with one or more nucleic acid probes which comprise said gene sequence or a mutant sequence of said gene;

(n) immunoblotting;

(o) immunocytochemistry;

(p) assaying for binding interactions between said gene protein isolated from said tissue and a binding partner capable of specifically binding the polypeptide expression product of a mutant allele and/or a binding partner for the polypeptide; and

(q) assaying for the inhibition of biochemical activity of said binding partner.

12. A nucleic acid probe which hybridizes to the isolated DNA of claim 1 under conditions at which it will not hybridize to wild-type DNA.

13. A method for diagnosing a mutation which causes long QT syndrome comprising hybridizing a probe of claim 12 to a patient's sample of DNA or RNA, the presence of a hybridization signal being indicative of long QT syndrome.

14. A method according to claim 13 wherein the patient's DNA or RNA has been amplified and said amplified DNA or RNA is hybridized with a probe of claim 12.

15. A method according to claim 13 wherein said hybridization is performed *in situ*.

16. A method according to claim 13 wherein said assay is performed using nucleic acid microchip technology.

17. A method for diagnosing a mutation which causes long QT syndrome comprising amplifying a region of gene or RNA for *KVLQT1* and sequencing the amplified gene or RNA wherein long QT syndrome is indicated by any one or more mutations selected from the group consisting of A332G, G478A, G521A, G535A, G580C, C727T, T742C, T797C, G921+1T, A922-2C, G928A, C1046G, C1066T, G1097A, C1172T, C1343G, C1588T, C1697T, C1747T and G1781A.

18. A method for diagnosing a mutation which causes long QT syndrome comprising identifying a mismatch between a patient's DNA or RNA and a wild-type DNA or RNA probe wherein said probe hybridizes to a region of DNA or RNA wherein said region comprises a mutation of SEQ ID NO:1 selected from the group consisting of A332G,

G478A, G521A, G535A, G580C, C727T, T742C, T797C, G921+1T, A922-2C, G928A, C1046G, C1066T, G1097A, C1172T, C1343G, C1588T, C1697T, C1747T and G1781A.

19. The method of claim 18 wherein the mismatch is identified by an RNase assay.

20. A method for diagnosing long QT syndrome said method consisting of an assay for the presence of mutant KVLQT1 polypeptide in a patient by reacting a patient's sample with an antibody of claim 7, the presence of a positive reaction being indicative of long QT syndrome.

21. The method of claim 20 wherein said assay comprises immunoblotting.

22. The method of claim 20 wherein said assay comprises an immunocytochemical technique.

23. A method for diagnosing long QT syndrome, said method comprising analyzing a KVLQT1 polypeptide, a mutation in said polypeptide being indicative of long QT syndrome wherein said mutation is a mutation selected from the group consisting of Y111C, E160K, R174H, G179S, A194P, R243C, W248R, L266P, V307sp, V310I, S349W, Q356X, R366Q, T391I, P448R, Q530X, S566F, R583C and R594Q.

24. A method to screen for drugs which are useful in treating a person with a mutation in *KVLQT1* wherein said mutation is selected from the group consisting of A332G, G478A, G521A, G535A, G580C, C727T, T742C, T797C, G921+1T, A922-2C, G928A, C1046G, C1066T, G1097A, C1172T, C1343G, C1588T, C1697T, C1747T and G1781A, said method comprising:

a) placing a first set of cells expressing KVLQT1 with a mutation, wherein said mutation is selected from the group consisting of Y111C, E160K, R174H, G179S, A194P, R243C, W248R, L266P, V307sp, V310I, S349W, Q356X, R366Q, T391I, P448R, Q530X, S566F, R583C and R594Q, into a bathing solution;

b) inducing a first induced K^+ current in the cells of step (a);

c) measuring said first induced K^+ current;

d) placing a second set of cells expressing wild-type KVLQT1 into a bathing solution

e) inducing a second induced K^+ current in the cells of step (d);

f) measuring said second induced K^+ current;

g) adding a drug to the bathing solution of step (a);

h) inducing a third induced K^+ current in the cells of step (g);

i) measuring said third induced K^+ current; and

j) determining whether the third induced K^+ current is more similar to the second induced K^+ current than is the first induced K^+ current, wherein drugs resulting in a third induced K^+ current which is closer to the second induced K^+ current than is the first induced K^+ current are useful in treating said persons.

25. An isolated DNA encoding a KVLQT1 polypeptide of SEQ ID NO:2 having a mutation selected from the group consisting of Y111C, E160K, R174H, G179S, A194P, R243C, W248R, L266P, V307sp, V310I, S349W, Q356X, R366Q, T391I, P448R, Q530X, S566F, R583C and R594Q.

26. An isolated DNA comprising a sequence of SEQ ID NO:3 as altered by one or more mutations selected from the group consisting of G3340A, C4501G, del4850-4852, G4868T, G5349A and G5360A.

27. A nucleic acid probe specifically hybridizable to a human mutated *SCN5A* and not to wild-type DNA, said mutated *SCN5A* comprising a mutation of SEQ ID NO:3 selected from the group consisting of G3340A, C4501G, del4850-4852, G4868T, G5349A and G5360A.

28. A method for detecting a mutation in *SCN5A* said mutation selected from the group consisting of G3340A, C4501G, del4850-4852, G4868T, G5349A and G5360A which comprises analyzing a sequence of said gene or RNA from a human sample or analyzing the sequence of cDNA made from mRNA from said sample.

29. The method of claim 28 wherein said mutation is detected by a method selected from the group consisting of:

a) hybridizing a probe specific for one of said mutations to RNA isolated from said human sample and detecting the presence of a hybridization product, wherein the presence of said product indicates the presence of said mutation in the sample;

b) hybridizing a probe specific for one of said mutations to cDNA made from RNA isolated from said sample and detecting the presence of a hybridization product, wherein the presence of said product indicates the presence of said mutation in the sample;

c) hybridizing a probe specific for one of said mutations to genomic DNA isolated from said sample and detecting the presence of a hybridization product, wherein the presence of said product indicates the presence of said mutation in the sample;

d) amplifying all or part of said gene in said sample using a set of primers to produce amplified nucleic acids and sequencing the amplified nucleic acids;

e) amplifying part of said gene in said sample using a primer specific for one of said mutations and detecting the presence of an amplified product, wherein the presence of said product indicates the presence of said mutation in the sample;

f) molecularly cloning all or part of said gene in said sample to produce a cloned nucleic acid and sequencing the cloned nucleic acid;

g) amplifying said gene to produce amplified nucleic acids, hybridizing the amplified nucleic acids to a DNA probe specific for one of said mutations and detecting the presence of a hybridization product, wherein the presence of said product indicates the presence of said mutation;

h) forming single-stranded DNA from a gene fragment of said gene from said human sample and single-stranded DNA from a corresponding fragment of a wild-type gene, electrophoresing said single-stranded DNAs on a non-denaturing polyacrylamide gel and comparing the mobility of said single-stranded DNAs on said gel to determine if said single-stranded DNA from said sample is shifted relative to wild-type and sequencing said single-stranded DNA having a shift in mobility;

i) forming a heteroduplex consisting of a first strand of nucleic acid selected from the group consisting of a genomic DNA fragment isolated from said sample, an RNA fragment isolated from said sample and a cDNA fragment made from mRNA from said

sample and a second strand of a nucleic acid consisting of a corresponding human wild-type gene fragment, analyzing for the presence of a mismatch in said heteroduplex, and sequencing said first strand of nucleic acid having a mismatch;

j) forming single-stranded DNA from said gene of said human sample and from a corresponding fragment of an allele specific for one of said mutations, electrophoresing said single-stranded DNAs on a non-denaturing polyacrylamide gel and comparing the mobility of said single-stranded DNAs on said gel to determine if said single-stranded DNA from said sample is shifted relative to said allele, wherein no shift in electrophoretic mobility of the single-stranded DNA relative to the allele indicates the presence of said mutation in said sample; and

k) forming a heteroduplex consisting of a first strand of nucleic acid selected from the group consisting of a genomic DNA fragment of said gene isolated from said sample, an RNA fragment isolated from said sample and a cDNA fragment made from mRNA from said sample and a second strand of a nucleic acid consisting of a corresponding gene allele fragment specific for one of said mutations and analyzing for the presence of a mismatch in said heteroduplex, wherein no mismatch indicates the presence of said mutation.

30. A method according to claim 29 wherein hybridization is performed *in situ*.

31. An isolated human polypeptide encoded by *SCN5A* comprising a mutation of SEQ ID NO:4 selected from the group consisting of D1114N, L1501V, delF1617, R1623L, E1784K and S1787N.

32. An antibody capable of binding the polypeptide of claim 31 but incapable of binding a wild-type polypeptide.

33. An antibody according to claim 32 wherein said antibody is a monoclonal antibody.

34. A method of assessing a risk in a human subject for long QT syndrome which comprises screening said subject for a mutation in *SCN5A* by comparing the sequence of said

SCN5A or its expression products isolated from a tissue sample of said subject with a wild-type sequence of said *SCN5A* or its expression products, wherein a mutation in the sequence of the subject indicates a risk for long QT syndrome.

35. The method of claim 34 wherein said expression product is selected from mRNA of said gene or a polypeptide encoded by said gene.

36. The method of claim 34 wherein one or more of the following procedures is carried out:
(a) observing shifts in electrophoretic mobility of single-stranded DNA from said sample on non-denaturing polyacrylamide gels;
(b) hybridizing a probe to genomic DNA isolated from said sample under conditions suitable for hybridization of said probe to said gene;
(c) determining hybridization of an allele-specific probe to genomic DNA from said sample;
(d) amplifying all or part of said gene from said sample to produce an amplified sequence and sequencing the amplified sequence;
(e) determining by nucleic acid amplification the presence of a specific mutant allele in said sample;
(f) molecularly cloning all or part of said gene from said sample to produce a cloned sequence and sequencing the cloned sequence;
(g) determining whether there is a mismatch between molecules (1) said gene genomic DNA or mRNA isolated from said sample, and (2) a nucleic acid probe complementary to the human wild-type gene DNA, when molecules (1) and (2) are hybridized to each other to form a duplex;
(h) amplification of said gene sequences in said sample and hybridization of the amplified sequences to nucleic acid probes which comprise wild-type gene sequences;
(i) amplification of said gene sequences in said tissue and hybridization of the amplified sequences to nucleic acid probes which comprise said mutant gene sequences;
(j) screening for a deletion mutation;
(k) screening for a point mutation;
(l) screening for an insertion mutation;

(m) determining *in situ* hybridization of said gene in said sample with one or more nucleic acid probes which comprise said gene sequence or a mutant sequence of said gene;

(n) immunoblotting;

(o) immunocytochemistry;

(p) assaying for binding interactions between said gene protein isolated from said tissue and a binding partner capable of specifically binding the polypeptide expression product of a mutant allele and/or a binding partner for the polypeptide; and

(q) assaying for the inhibition of biochemical activity of said binding partner.

37. A nucleic acid probe which hybridizes to the isolated DNA of claim 26 under conditions at which it will not hybridize to wild-type DNA.

38. A method for diagnosing a mutation which causes long QT syndrome comprising hybridizing a probe of claim 37 to a patient's sample of DNA or RNA, the presence of a hybridization signal being indicative of long QT syndrome.

39. A method according to claim 38 wherein the patient's DNA or RNA has been amplified and said amplified DNA or RNA is hybridized with a probe of claim 37.

40. A method according to claim 38 wherein said hybridization is performed *in situ*.

41. A method according to claim 38 wherein said assay is performed using nucleic acid microchip technology.

42. A method for diagnosing a mutation which causes long QT syndrome comprising amplifying a region of gene or RNA for *SCN5A* and sequencing the amplified gene or RNA wherein long QT syndrome is indicated by any one or more mutations selected from the group consisting of G3340A, C4501G, del4850-4852, G4868T, G5349A and G5360A.

43. A method for diagnosing a mutation which causes long QT syndrome comprising identifying a mismatch between a patient's DNA or RNA and a wild-type DNA or RNA probe wherein said probe hybridizes to a region of DNA or RNA wherein said region comprises a mutation of SEQ ID NO:3 selected from the group consisting of G3340A, C4501G, del4850-4852, G4868T, G5349A and G5360A.

44. The method of claim 43 wherein the mismatch is identified by an RNase assay.

45. A method for diagnosing long QT syndrome said method consisting of an assay for the presence of mutant SCN5A polypeptide in a patient by reacting a patient's sample with an antibody of claim 32, the presence of a positive reaction being indicative of long QT syndrome.

46. The method of claim 45 wherein said assay comprises immunoblotting.

47. The method of claim 45 wherein said assay comprises an immunocytochemical technique.

48. A method for diagnosing long QT syndrome, said method comprising analyzing a SCN5A polypeptide, a mutation in said polypeptide being indicative of long QT syndrome wherein said mutation is a mutation selected from the group consisting of D1114N, L1501V, delF1617, R1623L, E1784K and S1787N.

49. A method to screen for drugs which are useful in treating a person with a mutation in *SCN5A* wherein said mutation is selected from the group consisting of G3340A, C4501G, del4850-4852, G4868T, G5349A and G5360A, said method comprising:

a) placing a first set of cells expressing SCN5A with a mutation, wherein said mutation is selected from the group consisting of D1114N, L1501V, delF1617, R1623L, E1784K and S1787N, into a bathing solution;

b) inducing a first induced Na^+ current in the cells of step (a);

c) measuring said first induced Na^+ current;

d) placing a second set of cells expressing wild-type SCN5A into a bathing solution;

e) inducing a second induced Na^+ current in the cells of step (d);

f) measuring said second induced Na^+ current;

g) adding a drug to the bathing solution of step (a);

h) inducing a third induced Na^+ current in the cells in step (g);

i) measuring said third induced Na^+ current; and

j) determining whether the third induced Na^+ current is more similar to the second induced Na^+ current than is the first induced Na^+ current, wherein drugs resulting in a third induced Na^+ current which is closer to the second induced Na^+ current than is the first induced Na^+ current are useful in treating said persons.

50. An isolated DNA encoding an SCN5A polypeptide of SEQ ID NO:4 having a mutation selected from the group consisting of D1114N, L1501V, delF1617, R1623L, E1784K and S1787N.

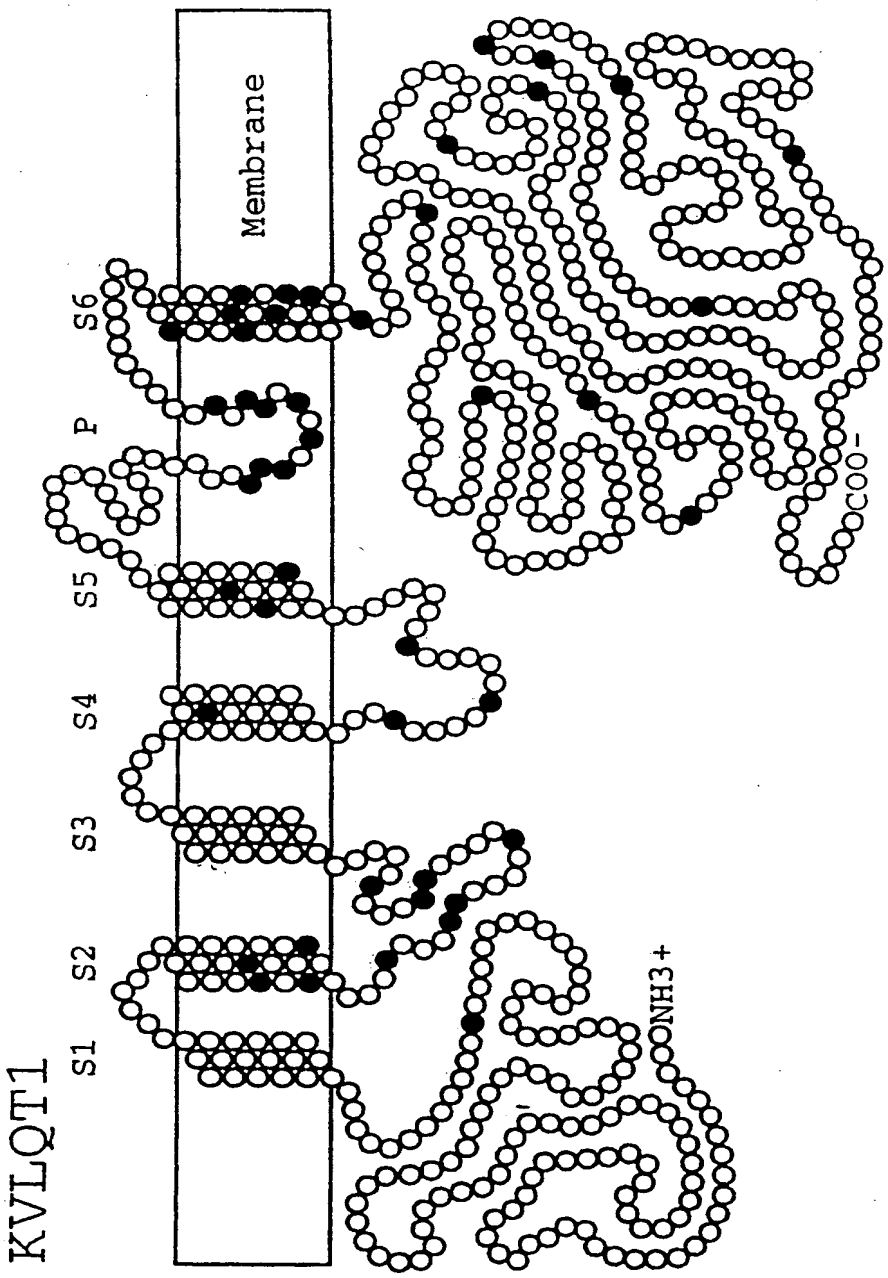

FIG. 1

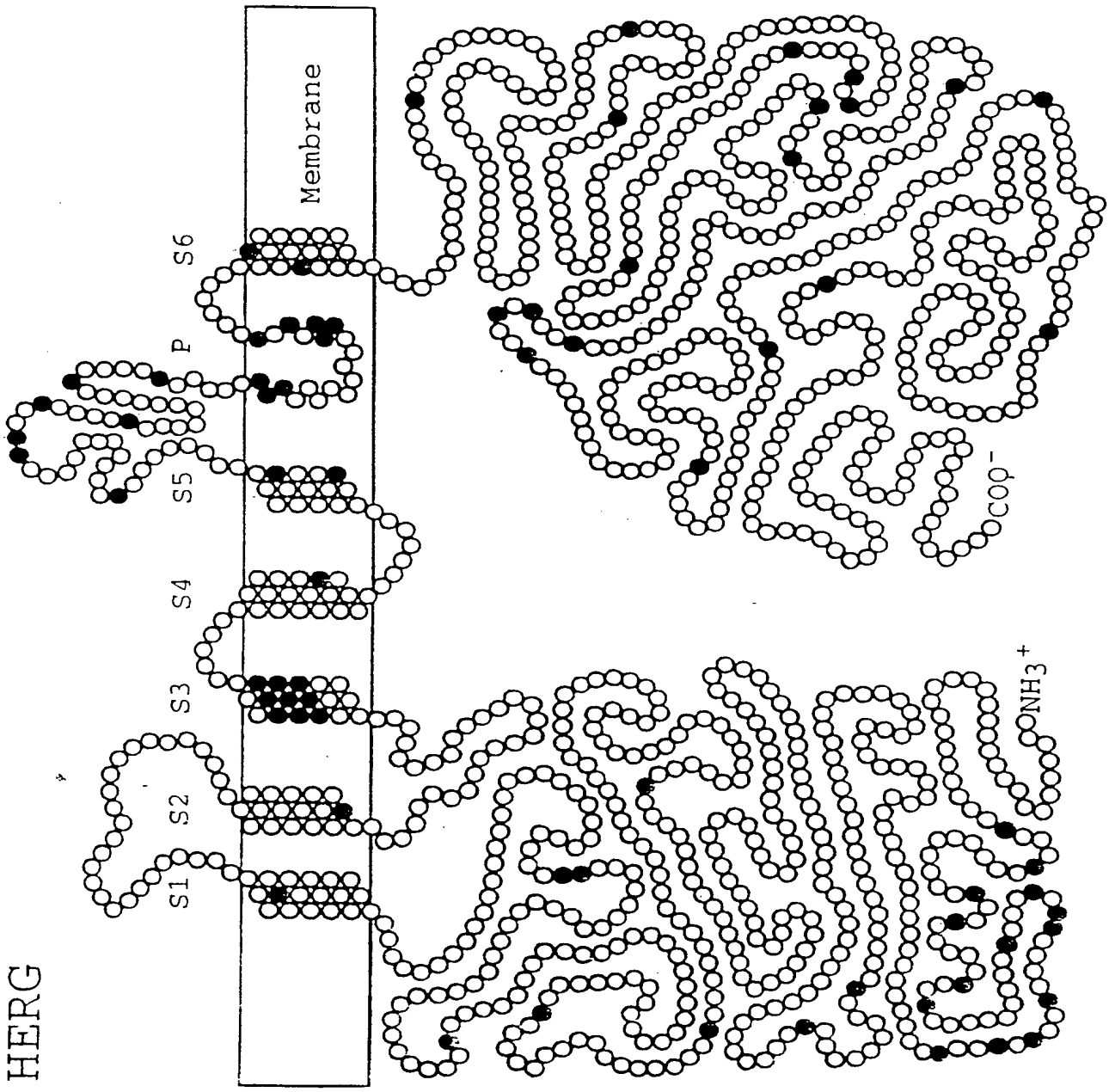

FIG. 2

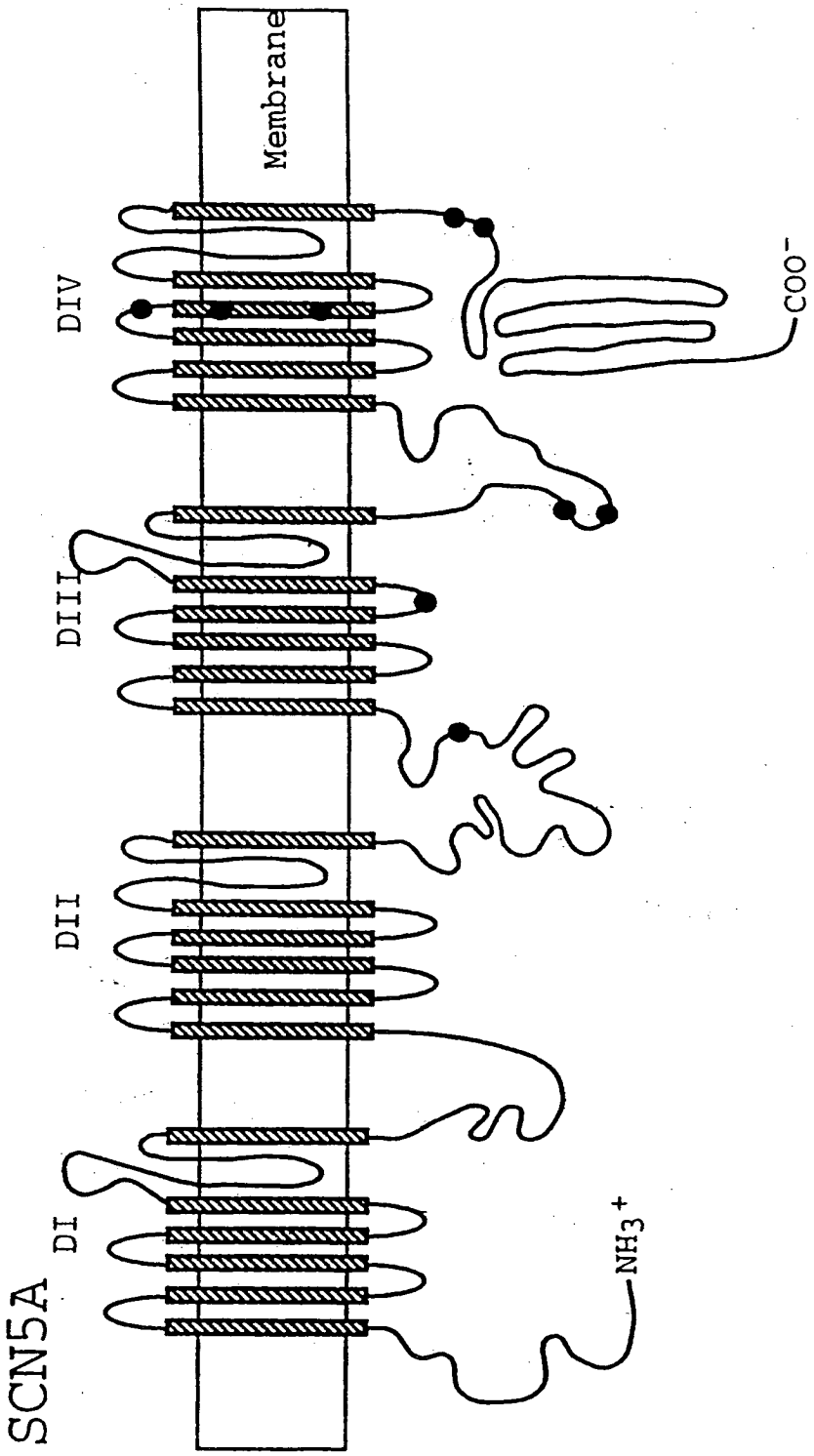

FIG. 3

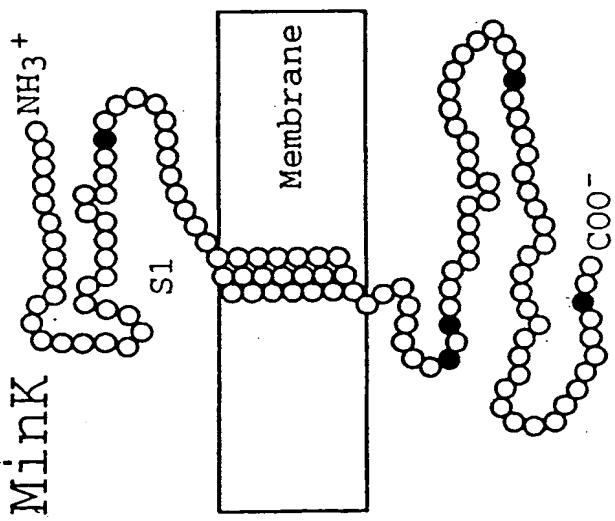

FIG. 4

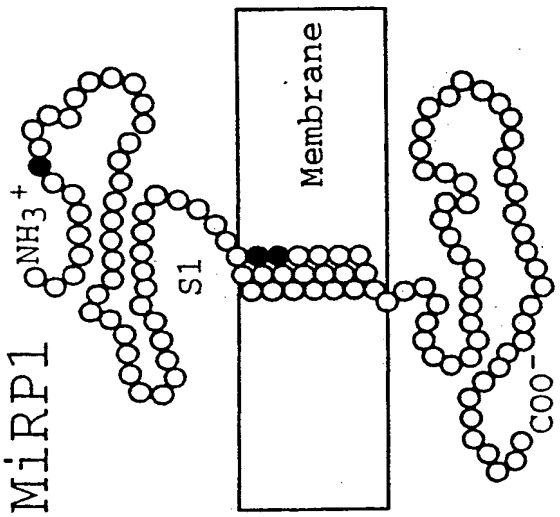

FIG.5

SEQUENCE LISTING

<110> Splawski, Igor
Keating, Mark T.
University of Utah Research Foundation

<120> ALTERATIONS IN THE LONG QT SYNDROME GENES KVLQT1 AND SCN5A AND METHODS FOR DETECTING SAME

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Trp Gly Arg Leu Pro Gly Ala Arg Arg Gly Ser Ala Gly Leu Ala Lys
             20                  25                  30

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gcg tcc ccg gcc gcg ccc gcc gcg ccc cca gtt gcc tcc gac ctt ggc   240
Ala Ser Pro Ala Ala Pro Ala Ala Pro Pro Val Ala Ser Asp Leu Gly
 65                  70                  75                  80

ccg cgg ccg ccg gtg agc cta gac ccg cgc gtc tcc atc tac agc acg   288
Pro Arg Pro Pro Val Ser Leu Asp Pro Arg Val Ser Ile Tyr Ser Thr
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ttt gcc cgg aag ccc att tcc atc atc gac ctc atc gtg gtc gtg gcc   624
Phe Ala Arg Lys Pro Ile Ser Ile Ile Asp Leu Ile Val Val Val Ala
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            260                 265                 270

ctc atc ttc tcc tcg tac ttt gtg tac ctg gct gag aag gac gcg gtg   864
Leu Ile Phe Ser Ser Tyr Phe Val Tyr Leu Ala Glu Lys Asp Ala Val
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aac gag tca ggc cgc gtg gag ttc ggc agc tac gca gat gcg ctg tgg   912
Asn Glu Ser Gly Arg Val Glu Phe Gly Ser Tyr Ala Asp Ala Leu Trp
    290                 295                 300

tgg ggg gtg gtc aca gtc acc acc atc ggc tat ggg gac aag gtg ccc   960
Trp Gly Val Val Thr Val Thr Thr Ile Gly Tyr Gly Asp Lys Val Pro
305                 310                 315                 320

cag acg tgg gtc ggg aag acc atc gcc tcc tgc ttc tct gtc ttt gcc  1008
Gln Thr Trp Val Gly Lys Thr Ile Ala Ser Cys Phe Ser Val Phe Ala
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atc tcc ttc ttt gcg ctc cca gcg ggg att ctt ggc tcg ggg ttt gcc  1056
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Glu Asn Pro Asp Ser Ser Thr Trp Lys Ile Tyr Ile Arg Lys Ala Pro
385                 390                 395                 400

cgg agc cac act ctg ctg tca ccc agc ccc aaa ccc aag aag tct gtg   1248
Arg Ser His Thr Leu Leu Ser Pro Ser Pro Lys Pro Lys Lys Ser Val
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gtg gta aag aaa aaa aag ttc aag ctg gac aaa gac aat ggg gtg act   1296
Val Val Lys Lys Lys Lys Phe Lys Leu Asp Lys Asp Asn Gly Val Thr
            420                 425                 430

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Pro Gly Glu Lys Met Leu Thr Val Pro His Ile Thr Cys Asp Pro Pro
        435                 440                 445

gaa gag cgg cgg ctg gac cac ttc tct gtc gac ggc tat gac agt tct   1392
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ctg aca ccc atc acc cac atc tca cag ctg cgg gaa cac cat cgg gcc   1536
Leu Thr Pro Ile Thr His Ile Ser Gln Leu Arg Glu His His Arg Ala
            500                 505                 510

acc att aag gtc att cga cgc atg cag tac ttt gtg gcc aag aag aaa   1584
Thr Ile Lys Val Ile Arg Arg Met Gln Tyr Phe Val Ala Lys Lys Lys
        515                 520                 525

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agg agg ctg gac cag tcc att ggg aag ccc tca ctg ttc atc tcc gtc   1728
Arg Arg Leu Asp Gln Ser Ile Gly Lys Pro Ser Leu Phe Ile Ser Val
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Asn Arg Val Glu Asp Lys Val Thr Gln Leu Asp Gln Arg Leu Ala Leu
        595                 600                 605

Ile Thr Asp Met Leu His Gln Leu Leu Ser Leu His Gly Gly Ser Thr
    610                 615                 620

Pro Gly Ser Gly Gly Pro Pro Arg Glu Gly Gly Ala His Ile Thr Gln
625                 630                 635                 640

Pro Cys Gly Ser Gly Gly Ser Val Asp Pro Glu Leu Phe Leu Pro Ser
                645                 650                 655

Asn Thr Leu Pro Thr Tyr Glu Gln Leu Thr Val Pro Arg Arg Gly Pro
            660                 665                 670

Asp Glu Gly Ser
        675
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Met Ala Asn Phe Leu Leu Pro Arg Gly Thr Ser Ser Phe Arg Arg Phe
  1               5                  10                  15

aca cgg gag tcc ctg gca gcc atc gag aag cgc atg gcg gag aag caa   96
Thr Arg Glu Ser Leu Ala Ala Ile Glu Lys Arg Met Ala Glu Lys Gln
            20                  25                  30

gcc cgc ggc tca acc acc ttg cag gag agc cga gag ggg ctg ccc gag  144
Ala Arg Gly Ser Thr Thr Leu Gln Glu Ser Arg Glu Gly Leu Pro Glu
        35                  40                  45

gag gag gct ccc cgg ccc cag ctg gac ctg cag gcc tcc aaa aag ctg  192
Glu Glu Ala Pro Arg Pro Gln Leu Asp Leu Gln Ala Ser Lys Lys Leu
    50                  55                  60

cca gat ctc tat ggc aat cca ccc caa gag ctc atc gga gag ccc ctg  240
Pro Asp Leu Tyr Gly Asn Pro Pro Gln Glu Leu Ile Gly Glu Pro Leu
65                  70                  75                  80

gag gac ctg gac ccc ttc tat agc acc caa aag act ttc atc gta ctg  288
Glu Asp Leu Asp Pro Phe Tyr Ser Thr Gln Lys Thr Phe Ile Val Leu
                85                  90                  95
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aat aaa ggc aag acc atc ttc cgg ttc agt gcc acc aac gcc ttg tat   336
Asn Lys Gly Lys Thr Ile Phe Arg Phe Ser Ala Thr Asn Ala Leu Tyr
            100                 105                 110

gtc ctc agt ccc ttc cac cca gtt cgg aga gcg gct gtg aag att ctg   384
Val Leu Ser Pro Phe His Pro Val Arg Arg Ala Ala Val Lys Ile Leu
        115                 120                 125

gtt cac tcg ctc ttc aac atg ctc atc atg tgc acc atc ctc acc aac   432
Val His Ser Leu Phe Asn Met Leu Ile Met Cys Thr Ile Leu Thr Asn
    130                 135                 140

tgc gtg ttc atg gcc cag cac gac cct cca ccc tgg acc aag tat gtc   480
Cys Val Phe Met Ala Gln His Asp Pro Pro Pro Trp Thr Lys Tyr Val
145                 150                 155                 160

gag tac acc ttc acc gcc att tac acc ttt gag tct ctg gtc aag att   528
Glu Tyr Thr Phe Thr Ala Ile Tyr Thr Phe Glu Ser Leu Val Lys Ile
                165                 170                 175

ctg gct cga gct ttc tgc ctg cac gcg ttc act ttc ctt cgg gac cca   576
Leu Ala Arg Ala Phe Cys Leu His Ala Phe Thr Phe Leu Arg Asp Pro
            180                 185                 190

tgg aac tgg ctg gac ttt agt gtg att atc atg gca tac aca act gaa   624
Trp Asn Trp Leu Asp Phe Ser Val Ile Ile Met Ala Tyr Thr Thr Glu
        195                 200                 205

ttt gtg gac ctg ggc aat gtc tca gcc tta cgc acc ttc cga gtc ctc   672
Phe Val Asp Leu Gly Asn Val Ser Ala Leu Arg Thr Phe Arg Val Leu
    210                 215                 220

cgg gcc ctg aaa act ata tca gtc att tca ggg ctg aag acc atc gtg   720
Arg Ala Leu Lys Thr Ile Ser Val Ile Ser Gly Leu Lys Thr Ile Val
225                 230                 235                 240

ggg gcc ctg atc cag tct gtg aag aag ctg gct gat gtg atg gtc ctc   768
Gly Ala Leu Ile Gln Ser Val Lys Lys Leu Ala Asp Val Met Val Leu
                245                 250                 255

aca gtc ttc tgc ctc agc gtc ttt gcc ctc atc ggc ctg cag ctc ttc   816
Thr Val Phe Cys Leu Ser Val Phe Ala Leu Ile Gly Leu Gln Leu Phe
            260                 265                 270

atg ggc aac cta agg cac aag tgt gtg cgc aac ttc aca gcg ctc aac   864
Met Gly Asn Leu Arg His Lys Cys Val Arg Asn Phe Thr Ala Leu Asn
        275                 280                 285

ggc acc aac ggc tcc gtg gag gcc gac ggc ttg gtc tgg gaa tcc ctg   912
Gly Thr Asn Gly Ser Val Glu Ala Asp Gly Leu Val Trp Glu Ser Leu
    290                 295                 300

gac ctt tac ctc agt gat cca gaa aat tac ctg ctc aag aac ggc acc   960
Asp Leu Tyr Leu Ser Asp Pro Glu Asn Tyr Leu Leu Lys Asn Gly Thr
305                 310                 315                 320

tct gat gtg tta ctg tgt ggg aac agc tct gac gct ggg aca tgt ccg   1008
Ser Asp Val Leu Leu Cys Gly Asn Ser Ser Asp Ala Gly Thr Cys Pro
                325                 330                 335

gag ggc tac cgg tgc cta aag gca ggc gag aac ccc gac cac ggc tac   1056
Glu Gly Tyr Arg Cys Leu Lys Ala Gly Glu Asn Pro Asp His Gly Tyr
            340                 345                 350
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acc agc ttc gat tcc ttt gcc tgg gcc ttt ctt gca ctc ttc cgc ctg   1104
Thr Ser Phe Asp Ser Phe Ala Trp Ala Phe Leu Ala Leu Phe Arg Leu
        355                 360                 365

atg acg cag gac tgc tgg gag cgc ctc tat cag cag acc ctc agg tcc   1152
Met Thr Gln Asp Cys Trp Glu Arg Leu Tyr Gln Gln Thr Leu Arg Ser
    370                 375                 380

gca ggg aag atc tac atg atc ttc ttc atg ctt gtc atc ttc ctg ggg   1200
Ala Gly Lys Ile Tyr Met Ile Phe Phe Met Leu Val Ile Phe Leu Gly
385                 390                 395                 400

tcc ttc tac ctg gtg aac ctg atc ctg gcc gtg gtc gca atg gcc tat   1248
Ser Phe Tyr Leu Val Asn Leu Ile Leu Ala Val Val Ala Met Ala Tyr
                405                 410                 415

gag gag caa aac caa gcc acc atc gct gag acc gag gag aag gaa aag   1296
Glu Glu Gln Asn Gln Ala Thr Ile Ala Glu Thr Glu Glu Lys Glu Lys
            420                 425                 430

cgc ttc cag gag gcc atg gaa atg ctc aag aaa gaa cac gag gcc ctc   1344
Arg Phe Gln Glu Ala Met Glu Met Leu Lys Lys Glu His Glu Ala Leu
        435                 440                 445

acc atc agg ggt gtg gat acc gtg tcc cgt agc tcc ttg gag atg tcc   1392
Thr Ile Arg Gly Val Asp Thr Val Ser Arg Ser Ser Leu Glu Met Ser
    450                 455                 460

cct ttg gcc cca gta aac agc cat gag aga aga agc aag agg aga aaa   1440
Pro Leu Ala Pro Val Asn Ser His Glu Arg Arg Ser Lys Arg Arg Lys
465                 470                 475                 480

cgg atg tct tca gga act gag gag tgt ggg gag gac agg ctc ccc aag   1488
Arg Met Ser Ser Gly Thr Glu Glu Cys Gly Glu Asp Arg Leu Pro Lys
                485                 490                 495

tct gac tca gaa gat ggt ccc aga gca atg aat cat ctc agc ctc acc   1536
Ser Asp Ser Glu Asp Gly Pro Arg Ala Met Asn His Leu Ser Leu Thr
            500                 505                 510

cgt ggc ctc agc agg act tct atg aag cca cgt tcc agc cgc ggg agc   1584
Arg Gly Leu Ser Arg Thr Ser Met Lys Pro Arg Ser Ser Arg Gly Ser
        515                 520                 525

att ttc acc ttt cgc agg cga gac ctg ggt tct gaa gca gat ttt gca   1632
Ile Phe Thr Phe Arg Arg Arg Asp Leu Gly Ser Glu Ala Asp Phe Ala
    530                 535                 540

gat gat gaa aac agc aca gcg cgg gag agc gag agc cac cac aca tca   1680
Asp Asp Glu Asn Ser Thr Ala Arg Glu Ser Glu Ser His His Thr Ser
545                 550                 555                 560

ctg ctg gtg ccc tgg ccc ctg cgc cgg acc agt gcc cag gga cag ccc   1728
Leu Leu Val Pro Trp Pro Leu Arg Arg Thr Ser Ala Gln Gly Gln Pro
                565                 570                 575

agt ccc gga acc tcg gct cct ggc cac gcc ctc cat ggc aaa aag aac   1776
Ser Pro Gly Thr Ser Ala Pro Gly His Ala Leu His Gly Lys Lys Asn
            580                 585                 590

agc act gtg gac tgc aat ggg gtg gtc tca tta ctg ggg gca ggc gac   1824
Ser Thr Val Asp Cys Asn Gly Val Val Ser Leu Leu Gly Ala Gly Asp
        595                 600                 605
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cca gag gcc aca tcc cca gga agc cac ctc ctc cgc cct gtg atg cta   1872
Pro Glu Ala Thr Ser Pro Gly Ser His Leu Leu Arg Pro Val Met Leu
    610                 615                 620

gag cac ccg cca gac acg acc acg cca tcg gag gag cca ggc ggc ccc   1920
Glu His Pro Pro Asp Thr Thr Thr Pro Ser Glu Glu Pro Gly Gly Pro
625                 630                 635                 640

cag atg ctg acc tcc cag gct ccg tgt gta gat ggc ttc gag gag cca   1968
Gln Met Leu Thr Ser Gln Ala Pro Cys Val Asp Gly Phe Glu Glu Pro
                645                 650                 655

gga gca cgg cag cgg gcc ctc agc gca gtc agc gtc ctc aca agc gca   2016
Gly Ala Arg Gln Arg Ala Leu Ser Ala Val Ser Val Leu Thr Ser Ala
            660                 665                 670

ctg gaa gag tta gag gag tct cgc cac aag tgt cca cca tgc tgg aac   2064
Leu Glu Glu Leu Glu Glu Ser Arg His Lys Cys Pro Pro Cys Trp Asn
        675                 680                 685

cgt ctc gcc cag cgc tac ctg atc tgg gag tgc tgc ccg ctg tgg atg   2112
Arg Leu Ala Gln Arg Tyr Leu Ile Trp Glu Cys Cys Pro Leu Trp Met
    690                 695                 700

tcc atc aag cag gga gtg aag ttg gtg gtc atg gac ccg ttt act gac   2160
Ser Ile Lys Gln Gly Val Lys Leu Val Val Met Asp Pro Phe Thr Asp
705                 710                 715                 720

ctc acc atc act atg tgc atc gta ctc aac aca ctc ttc atg gcg ctg   2208
Leu Thr Ile Thr Met Cys Ile Val Leu Asn Thr Leu Phe Met Ala Leu
                725                 730                 735

gag cac tac aac atg aca agt gaa ttc gag gag atg ctg cag gtc gga   2256
Glu His Tyr Asn Met Thr Ser Glu Phe Glu Glu Met Leu Gln Val Gly
            740                 745                 750

aac ctg gtc ttc aca ggg att ttc aca gca gag atg acc ttc aag atc   2304
Asn Leu Val Phe Thr Gly Ile Phe Thr Ala Glu Met Thr Phe Lys Ile
        755                 760                 765

att gcc ctc gac ccc tac tac tac ttc caa cag ggc tgg aac atc ttc   2352
Ile Ala Leu Asp Pro Tyr Tyr Tyr Phe Gln Gln Gly Trp Asn Ile Phe
    770                 775                 780

gac agc atc atc gtc atc ctt agc ctc atg gag ctg ggc ctg tcc cgc   2400
Asp Ser Ile Ile Val Ile Leu Ser Leu Met Glu Leu Gly Leu Ser Arg
785                 790                 795                 800

atg agc aac ttg tcg gtg ctg cgc tcc ttc cgc ctg ctg cgg gtc ttc   2448
Met Ser Asn Leu Ser Val Leu Arg Ser Phe Arg Leu Leu Arg Val Phe
                805                 810                 815

aag ctg gcc aaa tca tgg ccc acc ctg aac aca ctc atc aag atc atc   2496
Lys Leu Ala Lys Ser Trp Pro Thr Leu Asn Thr Leu Ile Lys Ile Ile
            820                 825                 830

ggg aac tca gtg ggg gca ctg ggg aac ctg aca ctg gtg cta gcc atc   2544
Gly Asn Ser Val Gly Ala Leu Gly Asn Leu Thr Leu Val Leu Ala Ile
        835                 840                 845

atc gtg ttc atc ttt gct gtg gtg ggc atg cag ctc ttt ggc aag aac   2592
Ile Val Phe Ile Phe Ala Val Val Gly Met Gln Leu Phe Gly Lys Asn
    850                 855                 860
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tac tcg gag ctg agg gac agc gac tca ggc ctg ctg cct cgc tgg cac   2640
Tyr Ser Glu Leu Arg Asp Ser Asp Ser Gly Leu Leu Pro Arg Trp His
865                 870                 875                 880

atg atg gac ttc ttt cat gcc ttc cta atc atc ttc cgc atc ctc tgt   2688
Met Met Asp Phe Phe His Ala Phe Leu Ile Ile Phe Arg Ile Leu Cys
                885                 890                 895

gga gag tgg atc gag acc atg tgg gac tgc atg gag gtg tcg ggg cag   2736
Gly Glu Trp Ile Glu Thr Met Trp Asp Cys Met Glu Val Ser Gly Gln
            900                 905                 910

tca tta tgc ctg ctg gtc ttc ttg ctt gtt atg gtc att ggc aac ctt   2784
Ser Leu Cys Leu Leu Val Phe Leu Leu Val Met Val Ile Gly Asn Leu
        915                 920                 925

gtg gtc ctg aat ctc ttc ctg gcc ttg ctg ctc agc tcc ttc agt gca   2832
Val Val Leu Asn Leu Phe Leu Ala Leu Leu Leu Ser Ser Phe Ser Ala
    930                 935                 940

gac aac ctc aca gcc cct gat gag gac aga gag atg aac aac ctc cag   2880
Asp Asn Leu Thr Ala Pro Asp Glu Asp Arg Glu Met Asn Asn Leu Gln
945                 950                 955                 960

ctg gcc ctg gcc cgc atc cag agg ggc ctg cgc ttt gtc aag cgg acc   2928
Leu Ala Leu Ala Arg Ile Gln Arg Gly Leu Arg Phe Val Lys Arg Thr
                965                 970                 975

acc tgg gat ttc tgc tgt ggt ctc ctg cgg cac cgg cct cag aag ccc   2976
Thr Trp Asp Phe Cys Cys Gly Leu Leu Arg His Arg Pro Gln Lys Pro
            980                 985                 990

gca gcc ctt gcc gcc cag ggc cag ctg ccc agc tgc att gcc acc ccc   3024
Ala Ala Leu Ala Ala Gln Gly Gln Leu Pro Ser Cys Ile Ala Thr Pro
        995                1000                1005

tac tcc ccg cca ccc cca gag acg gag aag gtg cct ccc acc cgc aag   3072
Tyr Ser Pro Pro Pro Pro Glu Thr Glu Lys Val Pro Pro Thr Arg Lys
   1010                1015                1020

gaa aca cag ttt gag gaa ggc gag caa cca ggc cag ggc acc ccc ggg   3120
Glu Thr Gln Phe Glu Glu Gly Glu Gln Pro Gly Gln Gly Thr Pro Gly
1025                1030                1035                1040

gat cca gag ccc gtg tgt gtg ccc atc gct gtg gcc gag tca gac aca   3168
Asp Pro Glu Pro Val Cys Val Pro Ile Ala Val Ala Glu Ser Asp Thr
                1045                1050                1055

gat gac caa gaa gag gat gag gag aac agc ctg ggc acg gag gag gag   3216
Asp Asp Gln Glu Glu Asp Glu Glu Asn Ser Leu Gly Thr Glu Glu Glu
            1060                1065                1070

tcc agc aag cag cag gaa tcc cag cct gtg tcc ggc tgg ccc aga ggc   3264
Ser Ser Lys Gln Gln Glu Ser Gln Pro Val Ser Gly Trp Pro Arg Gly
        1075                1080                1085

cct ccg gat tcc agg acc tgg agc cag gtg tca gcg act gcc tcc tct   3312
Pro Pro Asp Ser Arg Thr Trp Ser Gln Val Ser Ala Thr Ala Ser Ser
   1090                1095                1100

gag gcc gag gcc agt gca tct cag gcc gac tgg cgg cag cag tgg aaa   3360
Glu Ala Glu Ala Ser Ala Ser Gln Ala Asp Trp Arg Gln Gln Trp Lys
1105                1110                1115                1120
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gcg gaa ccc cag gcc cca ggg tgc ggt gag acc cca gag gac agt tgc   3408
Ala Glu Pro Gln Ala Pro Gly Cys Gly Glu Thr Pro Glu Asp Ser Cys
                1125                1130                1135

tcc gag ggc agc aca gca gac atg acc aac acc gct gag ctc ctg gag   3456
Ser Glu Gly Ser Thr Ala Asp Met Thr Asn Thr Ala Glu Leu Leu Glu
            1140                1145                1150

cag atc cct gac ctc ggc cag gat gtc aag gac cca gag gac tgc ttc   3504
Gln Ile Pro Asp Leu Gly Gln Asp Val Lys Asp Pro Glu Asp Cys Phe
        1155                1160                1165

act gaa ggc tgt gtc cgg cgc tgt ccc tgc tgt gcg gtg gac acc aca   3552
Thr Glu Gly Cys Val Arg Arg Cys Pro Cys Cys Ala Val Asp Thr Thr
    1170                1175                1180

cag gcc cca ggg aag gtc tgg tgg cgg ttg cgc aag acc tgc tac cac   3600
Gln Ala Pro Gly Lys Val Trp Trp Arg Leu Arg Lys Thr Cys Tyr His
1185                1190                1195                1200

atc gtg gag cac agc tgg ttc gag aca ttc atc atc ttc atg atc cta   3648
Ile Val Glu His Ser Trp Phe Glu Thr Phe Ile Ile Phe Met Ile Leu
                1205                1210                1215

ctc agc agt gga gcg ctg gcc ttc gag gac atc tac cta gag gag cgg   3696
Leu Ser Ser Gly Ala Leu Ala Phe Glu Asp Ile Tyr Leu Glu Glu Arg
            1220                1225                1230

aag acc atc aag gtt ctg ctt gag tat gcc gac aag atg ttc aca tat   3744
Lys Thr Ile Lys Val Leu Leu Glu Tyr Ala Asp Lys Met Phe Thr Tyr
        1235                1240                1245

gtc ttc gtg ctg gag atg ctg ctc aag tgg gtg gcc tac ggc ttc aag   3792
Val Phe Val Leu Glu Met Leu Leu Lys Trp Val Ala Tyr Gly Phe Lys
    1250                1255                1260

aag tac ttc acc aat gcc tgg tgc tgg ctc gac ttc ctc atc gta gac   3840
Lys Tyr Phe Thr Asn Ala Trp Cys Trp Leu Asp Phe Leu Ile Val Asp
1265                1270                1275                1280

gtc tct ctg gtc agc ctg gtg gcc aac acc ctg ggc ttt gcc gag atg   3888
Val Ser Leu Val Ser Leu Val Ala Asn Thr Leu Gly Phe Ala Glu Met
                1285                1290                1295

ggc ccc atc aag tca ctg cgg acg ctg cgt gca ctc cgt cct ctg aga   3936
Gly Pro Ile Lys Ser Leu Arg Thr Leu Arg Ala Leu Arg Pro Leu Arg
            1300                1305                1310

gct ctg tca cga ttt gag ggc atg agg gtg gtg gtc aat gcc ctg gtg   3984
Ala Leu Ser Arg Phe Glu Gly Met Arg Val Val Val Asn Ala Leu Val
        1315                1320                1325

ggc gcc atc ccg tcc atc atg aac gtc ctc ctc gtc tgc ctc atc ttc   4032
Gly Ala Ile Pro Ser Ile Met Asn Val Leu Leu Val Cys Leu Ile Phe
    1330                1335                1340

tgg ctc atc ttc agc atc atg ggc gtg aac ctc ttt gcg ggg aag ttt   4080
Trp Leu Ile Phe Ser Ile Met Gly Val Asn Leu Phe Ala Gly Lys Phe
1345                1350                1355                1360

ggg agg tgc atc aac cag aca gag gga gac ttg cct ttg aac tac acc   4128
Gly Arg Cys Ile Asn Gln Thr Glu Gly Asp Leu Pro Leu Asn Tyr Thr
                1365                1370                1375
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atc gtg aac aac aag agc cag tgt gag tcc ttg aac ttg acc gga gaa   4176
Ile Val Asn Asn Lys Ser Gln Cys Glu Ser Leu Asn Leu Thr Gly Glu
          1380                1385                1390

ttg tac tgg acc aag gtg aaa gtc aac ttt gac aac gtg ggg gcc ggg   4224
Leu Tyr Trp Thr Lys Val Lys Val Asn Phe Asp Asn Val Gly Ala Gly
      1395                1400                1405

tac ctg gcc ctt ctg cag gtg gca aca ttt aaa ggc tgg atg gac att   4272
Tyr Leu Ala Leu Leu Gln Val Ala Thr Phe Lys Gly Trp Met Asp Ile
  1410                1415                1420

atg tat gca gct gtg gac tcc agg ggg tat gaa gag cag cct cag tgg   4320
Met Tyr Ala Ala Val Asp Ser Arg Gly Tyr Glu Glu Gln Pro Gln Trp
1425                1430                1435                1440

gaa tac aac ctc tac atg tac atc tat ttt gtc att ttc atc atc ttt   4368
Glu Tyr Asn Leu Tyr Met Tyr Ile Tyr Phe Val Ile Phe Ile Ile Phe
              1445                1450                1455

ggg tct ttc ttc acc ctg aac ctc ttt att ggt gtc atc att gac aac   4416
Gly Ser Phe Phe Thr Leu Asn Leu Phe Ile Gly Val Ile Ile Asp Asn
          1460                1465                1470

ttc aac caa cag aag aaa aag tta ggg ggc cag gac atc ttc atg aca   4464
Phe Asn Gln Gln Lys Lys Lys Leu Gly Gly Gln Asp Ile Phe Met Thr
      1475                1480                1485

gag gag cag aag aag tac tac aat gcc atg aag aag ctg ggc tcc aag   4512
Glu Glu Gln Lys Lys Tyr Tyr Asn Ala Met Lys Lys Leu Gly Ser Lys
  1490                1495                1500

aag ccc cag aag ccc atc cca cgg ccc ctg aac aag tac cag ggc ttc   4560
Lys Pro Gln Lys Pro Ile Pro Arg Pro Leu Asn Lys Tyr Gln Gly Phe
1505                1510                1515                1520

ata ttc gac att gtg acc aag cag gcc ttt gac gtc acc atc atg ttt   4608
Ile Phe Asp Ile Val Thr Lys Gln Ala Phe Asp Val Thr Ile Met Phe
              1525                1530                1535

ctg atc tgc ttg aat atg gtg acc atg atg gtg gag aca gat gac caa   4656
Leu Ile Cys Leu Asn Met Val Thr Met Met Val Glu Thr Asp Asp Gln
          1540                1545                1550

agt cct gag aaa atc aac atc ttg gcc aag atc aac ctg ctc ttt gtg   4704
Ser Pro Glu Lys Ile Asn Ile Leu Ala Lys Ile Asn Leu Leu Phe Val
      1555                1560                1565

gcc atc ttc aca ggc gag tgt att gtc aag ctg gct gcc ctg cgc cac   4752
Ala Ile Phe Thr Gly Glu Cys Ile Val Lys Leu Ala Ala Leu Arg His
  1570                1575                1580

tac tac ttc acc aac agc tgg aat atc ttc gac ttc gtg gtt gtc atc   4800
Tyr Tyr Phe Thr Asn Ser Trp Asn Ile Phe Asp Phe Val Val Val Ile
1585                1590                1595                1600

ctc tcc atc gtg ggc act gtg ctc tcg gac atc atc cag aag tac ttc   4848
Leu Ser Ile Val Gly Thr Val Leu Ser Asp Ile Ile Gln Lys Tyr Phe
              1605                1610                1615

ttc tcc ccg acg ctc ttc cga gtc atc cgc ctg gcc cga ata ggc cgc   4896
Phe Ser Pro Thr Leu Phe Arg Val Ile Arg Leu Ala Arg Ile Gly Arg
          1620                1625                1630
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atc ctc aga ctg atc cga ggg gcc aag ggg atc cgc acg ctg ctc ttt   4944
Ile Leu Arg Leu Ile Arg Gly Ala Lys Gly Ile Arg Thr Leu Leu Phe
       1635                1640                1645

gcc ctc atg atg tcc ctg cct gcc ctc ttc aac atc ggg ctg ctg ctc   4992
Ala Leu Met Met Ser Leu Pro Ala Leu Phe Asn Ile Gly Leu Leu Leu
   1650                1655                1660

ttc ctc gtc atg ttc atc tac tcc atc ttt ggc atg gcc aac ttc gct   5040
Phe Leu Val Met Phe Ile Tyr Ser Ile Phe Gly Met Ala Asn Phe Ala
1665                1670                1675                1680

tat gtc aag tgg gag gct ggc atc gac gac atg ttc aac ttc cag acc   5088
Tyr Val Lys Trp Glu Ala Gly Ile Asp Asp Met Phe Asn Phe Gln Thr
                1685                1690                1695

ttc gcc aac agc atg ctg tgc ctc ttc cag atc acc acg tcg gcc ggc   5136
Phe Ala Asn Ser Met Leu Cys Leu Phe Gln Ile Thr Thr Ser Ala Gly
           1700                1705                1710

tgg gat ggc ctc ctc agc ccc atc ctc aac act ggg ccg ccc tac tgc   5184
Trp Asp Gly Leu Leu Ser Pro Ile Leu Asn Thr Gly Pro Pro Tyr Cys
       1715                1720                1725

gac ccc act ctg ccc aac agc aat ggc tct cgg ggg gac tgc ggg agc   5232
Asp Pro Thr Leu Pro Asn Ser Asn Gly Ser Arg Gly Asp Cys Gly Ser
   1730                1735                1740

cca gcc gtg ggc atc ctc ttc ttc acc acc tac atc atc atc tcc ttc   5280
Pro Ala Val Gly Ile Leu Phe Phe Thr Thr Tyr Ile Ile Ile Ser Phe
1745                1750                1755                1760

ctc atc gtg gtc aac atg tac att gcc atc atc ctg gag aac ttc agc   5328
Leu Ile Val Val Asn Met Tyr Ile Ala Ile Ile Leu Glu Asn Phe Ser
                1765                1770                1775

gtg gcc acg gag gag agc acc gag ccc ctg agt gag gac gac ttc gat   5376
Val Ala Thr Glu Glu Ser Thr Glu Pro Leu Ser Glu Asp Asp Phe Asp
           1780                1785                1790

atg ttc tat gag atc tgg gag aaa ttt gac cca gag gcc act cag ttt   5424
Met Phe Tyr Glu Ile Trp Glu Lys Phe Asp Pro Glu Ala Thr Gln Phe
       1795                1800                1805

att gag tat tcg gtc ctg tct gac ttt gcc gac gcc ctg tct gag cca   5472
Ile Glu Tyr Ser Val Leu Ser Asp Phe Ala Asp Ala Leu Ser Glu Pro
   1810                1815                1820

ctc cgt atc gcc aag ccc aac cag ata agc ctc atc aac atg gac ctg   5520
Leu Arg Ile Ala Lys Pro Asn Gln Ile Ser Leu Ile Asn Met Asp Leu
1825                1830                1835                1840

ccc atg gtg agt ggg gac cgc atc cat tgc atg gac att ctc ttt gcc   5568
Pro Met Val Ser Gly Asp Arg Ile His Cys Met Asp Ile Leu Phe Ala
                1845                1850                1855

ttc acc aaa agg gtc ctg ggg gag tct ggg gag atg gac gcc ctg aag   5616
Phe Thr Lys Arg Val Leu Gly Glu Ser Gly Glu Met Asp Ala Leu Lys
           1860                1865                1870

atc cag atg gag gag aag ttc atg gca gcc aac cca tcc aag atc tcc   5664
Ile Gln Met Glu Glu Lys Phe Met Ala Ala Asn Pro Ser Lys Ile Ser
       1875                1880                1885
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tac gag ccc atc acc acc aca ctc cgg cgc aag cac gaa gag gtg tcg   5712
Tyr Glu Pro Ile Thr Thr Thr Leu Arg Arg Lys His Glu Glu Val Ser
   1890                1895                1900

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1905               1910                1915                1920

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               1925                1930                1935

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           1940                1945                1950

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       1955                1960                1965

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Ala Arg Gly Ser Thr Thr Leu Gln Glu Ser Arg Glu Gly Leu Pro Glu
         35                  40                  45

Glu Glu Ala Pro Arg Pro Gln Leu Asp Leu Gln Ala Ser Lys Lys Leu
     50                  55                  60

Pro Asp Leu Tyr Gly Asn Pro Pro Gln Glu Leu Ile Gly Glu Pro Leu
 65                  70                  75                  80

Glu Asp Leu Asp Pro Phe Tyr Ser Thr Gln Lys Thr Phe Ile Val Leu
                 85                  90                  95

Asn Lys Gly Lys Thr Ile Phe Arg Phe Ser Ala Thr Asn Ala Leu Tyr
            100                 105                 110

Val Leu Ser Pro Phe His Pro Val Arg Arg Ala Ala Val Lys Ile Leu
        115                 120                 125
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Val His Ser Leu Phe Asn Met Leu Ile Met Cys Thr Ile Leu Thr Asn
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Cys Val Phe Met Ala Gln His Asp Pro Pro Pro Trp Thr Lys Tyr Val
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Glu Tyr Thr Phe Thr Ala Ile Tyr Thr Phe Glu Ser Leu Val Lys Ile
                165                 170                 175

Leu Ala Arg Ala Phe Cys Leu His Ala Phe Thr Phe Leu Arg Asp Pro
            180                 185                 190

Trp Asn Trp Leu Asp Phe Ser Val Ile Ile Met Ala Tyr Thr Thr Glu
        195                 200                 205

Phe Val Asp Leu Gly Asn Val Ser Ala Leu Arg Thr Phe Arg Val Leu
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Arg Ala Leu Lys Thr Ile Ser Val Ile Ser Gly Leu Lys Thr Ile Val
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Gly Ala Leu Ile Gln Ser Val Lys Lys Leu Ala Asp Val Met Val Leu
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Thr Val Phe Cys Leu Ser Val Phe Ala Leu Ile Gly Leu Gln Leu Phe
            260                 265                 270

Met Gly Asn Leu Arg His Lys Cys Val Arg Asn Phe Thr Ala Leu Asn
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Gly Thr Asn Gly Ser Val Glu Ala Asp Gly Leu Val Trp Glu Ser Leu
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Asp Leu Tyr Leu Ser Asp Pro Glu Asn Tyr Leu Leu Lys Asn Gly Thr
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Ser Asp Val Leu Leu Cys Gly Asn Ser Ser Asp Ala Gly Thr Cys Pro
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Glu Gly Tyr Arg Cys Leu Lys Ala Gly Glu Asn Pro Asp His Gly Tyr
            340                 345                 350

Thr Ser Phe Asp Ser Phe Ala Trp Ala Phe Leu Ala Leu Phe Arg Leu
        355                 360                 365

Met Thr Gln Asp Cys Trp Glu Arg Leu Tyr Gln Gln Thr Leu Arg Ser
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Ala Gly Lys Ile Tyr Met Ile Phe Phe Met Leu Val Ile Phe Leu Gly
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Ser Phe Tyr Leu Val Asn Leu Ile Leu Ala Val Val Ala Met Ala Tyr
                405                 410                 415

Glu Glu Gln Asn Gln Ala Thr Ile Ala Glu Thr Glu Glu Lys Glu Lys
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Arg Phe Gln Glu Ala Met Glu Met Leu Lys Lys Glu His Glu Ala Leu
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Thr Ile Arg Gly Val Asp Thr Val Ser Arg Ser Ser Leu Glu Met Ser
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Arg Gly Leu Ser Arg Thr Ser Met Lys Pro Arg Ser Ser Arg Gly Ser
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Leu Leu Val Pro Trp Pro Leu Arg Arg Thr Ser Ala Gln Gly Gln Pro
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Ser Thr Val Asp Cys Asn Gly Val Val Ser Leu Leu Gly Ala Gly Asp
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Pro Glu Ala Thr Ser Pro Gly Ser His Leu Leu Arg Pro Val Met Leu
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Glu His Pro Pro Asp Thr Thr Thr Pro Ser Glu Glu Pro Gly Gly Pro
625                 630                 635                 640

Gln Met Leu Thr Ser Gln Ala Pro Cys Val Asp Gly Phe Glu Glu Pro
                645                 650                 655

Gly Ala Arg Gln Arg Ala Leu Ser Ala Val Ser Val Leu Thr Ser Ala
            660                 665                 670

Leu Glu Glu Leu Glu Glu Ser Arg His Lys Cys Pro Pro Cys Trp Asn
        675                 680                 685

Arg Leu Ala Gln Arg Tyr Leu Ile Trp Glu Cys Cys Pro Leu Trp Met
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Ser Ile Lys Gln Gly Val Lys Leu Val Val Met Asp Pro Phe Thr Asp
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Glu His Tyr Asn Met Thr Ser Glu Phe Glu Glu Met Leu Gln Val Gly
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Asn Leu Val Phe Thr Gly Ile Phe Thr Ala Glu Met Thr Phe Lys Ile
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```

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820 825 830

Gly Asn Ser Val Gly Ala Leu Gly Asn Leu Thr Leu Val Leu Ala Ile
835 840 845

Ile Val Phe Ile Phe Ala Val Val Gly Met Gln Leu Phe Gly Lys Asn
850 855 860

Tyr Ser Glu Leu Arg Asp Ser Asp Ser Gly Leu Leu Pro Arg Trp His
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Tyr Val Lys Trp Glu Ala Gly Ile Asp Asp Met Phe Asn Phe Gln Thr
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1795 1800 1805

Ile Glu Tyr Ser Val Leu Ser Asp Phe Ala Asp Ala Leu Ser Glu Pro
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1825 1830 1835 1840

Pro Met Val Ser Gly Asp Arg Ile His Cys Met Asp Ile Leu Phe Ala
1845 1850 1855

Phe Thr Lys Arg Val Leu Gly Glu Ser Gly Glu Met Asp Ala Leu Lys
1860 1865 1870

Ile Gln Met Glu Glu Lys Phe Met Ala Ala Asn Pro Ser Lys Ile Ser
1875 1880 1885

Tyr Glu Pro Ile Thr Thr Thr Leu Arg Arg Lys His Glu Glu Val Ser
1890 1895 1900

Ala Met Val Ile Gln Arg Ala Phe Arg Arg His Leu Leu Gln Arg Ser
1905 1910 1915 1920

Leu Lys His Ala Ser Phe Leu Phe Arg Gln Gln Ala Gly Ser Gly Leu
1925 1930 1935

Ser Glu Glu Asp Ala Pro Glu Arg Glu Gly Leu Ile Ala Tyr Val Met
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Ser Glu Asn Phe Ser Arg Pro Leu Gly Pro Pro Ser Ser Ser Ser Ile
1955 1960 1965

Ser Ser Thr Ser Phe Pro Pro Ser Tyr Asp Ser Val Thr Arg Ala Thr
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1985 1990 1995 2000

Leu Ala Asp Phe Pro Pro Ser Pro Asp Arg Asp Arg Glu Ser Ile Val
2005 2010 2015

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(54) Title: ALTERATIONS IN THE LONG QT SYNDROME GENES *KVLQT1* AND *SCN5A* AND METHODS FOR DETECTING SAME

(57) Abstract: Long QT Syndrome (LQTS) is a cardiovascular disorder characterized by prolongation of the QT interval on electrocardiogram and presence of syncope, seizures and sudden death. Five genes have been implicated in Romano-Ward syndrome, the autosomal dominant form of LQTS. These genes are *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* and *KCNE2*. Mutations in *KVLQT1* and *KCNE1* also cause the Jervell and Lange-Nielsen syndrome, a form of LQTS associated with deafness, a phenotypic abnormality inherited in an autosomal recessive fashion. Mutational analyses were used to screen 262 unrelated individuals with LQTS for mutations in the five defined genes. A total of 134 mutations were observed of which eighty were novel.

TITLE OF THE INVENTION

ALTERATIONS IN THE LONG QT SYNDROME GENES *KVLQT1* AND *SCN5A* AND METHODS FOR DETECTING SAME

This application was made with Government support from NHLBI under Grant Nos. RO1-HL46401, RO1-HL33843, RO1-HL51618, P50-HL52338 and MO1-RR000064. The federal government may have certain rights in this invention.

BACKGROUND OF THE INVENTION

Long QT Syndrome (LQTS) is a cardiovascular disorder characterized by prolongation of the QT interval on electrocardiogram and presence of syncope, seizures and sudden death, usually in young, otherwise healthy individuals (Jervell and Lange-Nielsen, 1957; Romano et al., 1963; Ward, 1964). The clinical features of LQTS result from episodic ventricular tachyarrhythmias, such as *torsade de pointes* and ventricular fibrillation (Schwartz et al., 1975; Moss et al., 1991). Two inherited forms of LQTS exist. The more common form, Romano-Ward syndrome (RW), is not associated with other phenotypic abnormalities and is inherited as an autosomal dominant trait with variable penetrance (Roman et al., 1963; Ward, 1964). Jervell and Lange-Nielsen syndrome (JLN) is characterized by the presence of deafness, a phenotypic abnormality inherited as an autosomal recessive trait (Jervell and Lange-Nielsen, 1957). LQTS can also be acquired, usually as a result of pharmacologic therapy.

In previous studies, we mapped LQTS loci to chromosomes 11p15.5 (*LQT1*) (Keating et al., 1991), 7 q35-36 (*LQT2*) (Jiang et al., 1994) and *LQT3* to 3p21-24 (Jiang et al., 1994). A fourth locus (*LQT4*) was mapped to 4q25-27 (Schott et al., 1995). Five genes have been implicated in Romano-Ward syndrome, the autosomal dominant form of LQTS. These genes are *KVLQT1* (*LQT1*) (Wang Q. et al., 1996a), *HERG* (*LQT2*) (Curran et al., 1995), *SCN5A* (*LQT3*) (Wang et al., 1995a), and two genes located at 21q22 - *KCNE1* (*LQT5*) (Splawski et al., 1997a) and *KCNE2* (*LQT6*) (Abbott et al., 1999). Mutations in *KVLQT1* and *KCNE1* also cause the Jervell and Lange-Nielsen syndrome, a form of LQTS associated with deafness, a phenotypic abnormality inherited in an autosomal recessive fashion.

KVLQT1, *HERG*, *KCNE1* and *KCNE2* encode potassium channel subunits. Four KVLQT1 α-subunits assemble with minK (β-subunits encoded by *KCNE1*, stoichiometry is

unknown) to form I_{Ks} channels underlying the slowly activating delayed rectifier potassium current in the heart (Sanguinetti et al., 1996a; Barhanin et al., 1996). Four HERG α-subunits assemble with MiRP1 (encoded by *KCNE2*, stoichiometry unknown) to form I_{Kr} channels, which underlie the rapidly activating, delayed rectifier potassium current (Abbott et al., 1999). Mutant subunits lead to reduction of I_{Ks} or I_{Kr} by a loss-of-function mechanism, often with a dominant-negative effect (Chouabe et al., 1997; Shalaby et al., 1997; Wollnik et al., 1997; Sanguinetti et al., 1996b). *SCN5A* encodes the cardiac sodium channel that is responsible for I_{Na}, the sodium current in the heart (Gellens et al., 1992). LQTS-associated mutations in *SCN5A* cause a gain-of-function (Bennett et al., 1995; Dumaine et al., 1996). In the heart, reduced I_{Ks} or I_{Kr} or increased I_{Na} leads to prolongation of the cardiac action potential, lengthening of the QT interval and increased risk of arrhythmia. *KVLQT1* and *KCNE1* are also expressed in the inner ear (Neyroud et al., 1997; Vetter et al., 1996). Others and we demonstrated that complete loss of I_{Ks} causes the severe cardiac phenotype and deafness in JLN (Neyroud et al., 1997; Splawski et al., 1997b; Tyson et al., 1997; Schulze-Bahr et al., 1997).

Presymptomatic diagnosis of LQTS is currently based on prolongation of the QT interval on electrocardiogram. Genetic studies, however, have shown that diagnosis based solely on electrocardiogram is neither sensitive nor specific (Vincent et al., 1992; Priori et al., 1999). Genetic screening using mutational analysis can improve presymptomatic diagnosis. However, a comprehensive study identifying and cataloging all LQTS-associated mutations in all five genes has not been achieved. To determine the relative frequency of mutations in each gene, facilitate presymptomatic diagnosis and enable genotype-phenotype studies, we screened a pool of 262 unrelated individuals with LQTS for mutations in the five defined genes. The results of these studies are presented in the Examples below.

The present invention relates to alterations in the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* and *KCNE2* genes and methods for detecting such alterations.

The publications and other materials used herein to illuminate the background of the invention or provide additional details respecting the practice, are incorporated by reference, and for convenience are respectively grouped in the appended List of References.

The present invention is directed to alterations in genes and gene products associated with long QT syndrome and to a process for the diagnosis and prevention of LQTS. LQTS is

diagnosed in accordance with the present invention by analyzing the DNA sequence of the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* gene of an individual to be tested and comparing the respective DNA sequence to the known DNA sequence of the normal gene. Alternatively, these genes of an individual to be tested can be screened for mutations which cause LQTS. Prediction of LQTS will enable practitioners to prevent this disorder using existing medical therapy.

SUMMARY OF THE INVENTION

The present invention relates to alterations in the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* and *KCNE2* genes and methods for detecting such alterations. The alterations in the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* and *KCNE2* genes include mutations and polymorphisms. Included among the mutations are frameshift, nonsense, splice, regulatory and missense mutations. Any method which is capable of detecting the alterations described herein can be used. Such methods include, but are not limited to, DNA sequencing, allele-specific probing, mismatch detection, single stranded conformation polymorphism detection and allele-specific PCR amplification.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a schematic representation of the predicted topology of KVLQT1 and the locations of LQTS-associated mutations. KVLQT1 consists of six putative transmembrane segments (S1 to S6) and a pore (Pore) region. Each circle represents an amino acid. The approximate location of LQTS-associated mutations identified in our laboratory are shown with filled circles.

Figure 2 is a schematic representation of HERG mutations. HERG consists of six putative transmembrane segments (S1 to S6) and a pore (Pore) region. Location of LQTS-associated mutations are shown with filled circles.

Figure 3 is a schematic representation of SCN5A and locations of LQTS-associated mutations. SCN5A consists of four domain (DI to DIV), each of which has six putative

transmembrane segments (S1 to S6) and a pore (Pore) region. Location of LQTS-associated mutations identified in our laboratory are shown with filled circles.

Figure 4 is a schematic representation of minK and locations of LQT-associated mutations. MinK consists of one putative transmembrane domain (S1). The approximate location of LQTS-associated mutations identified in our laboratory are shown with filled circles.

Figure 5 is a schematic representation of the predicted topology of MiRP1 and locations of arrhythmia-associated mutations. MiRP1 consists of one putative transmembrane domain (S1). The approximate location of arrhythmia-associated mutations identified in our laboratory are shown with filled circles.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to alterations in the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* and *KCNE2* genes and methods for detecting such alterations. The alterations in the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* and *KCNE2* genes include mutations and polymorphisms. Included among the mutations are frameshift, nonsense, splice, regulatory and missense mutations. Any method which is capable of detecting the mutations and polymorphisms described herein can be used. Such methods include, but are not limited to, DNA sequencing, allele-specific probing, mismatch detection, single stranded conformation polymorphism detection and allele-specific PCR amplification.

KVLQT1, *HERG*, *SCN5A*, *KCNE1* and *KCNE2* mutations cause increased risk for LQTS. Many different mutations occur in *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* and *KCNE2*. In order to detect the presence of alterations in the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* and *KCNE2* genes, a biological sample such as blood is prepared and analyzed for the presence or absence of a given alteration of *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2*. In order to detect the increased risk for LQTS or for the lack of such increased risk, a biological sample is prepared and analyzed for the presence or absence of a mutant allele of *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2*. Results of these tests and interpretive information are returned to the health care provider for communication to the tested individual. Such diagnoses may be performed by diagnostic

laboratories or, alternatively, diagnostic kits are manufactured and sold to health care providers or to private individuals for self-diagnosis.

The presence of hereditary LQTS may be ascertained by testing any tissue of a human for mutations of the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* gene. For example, a person who has inherited a germline *HERG* mutation would be prone to develop LQTS. This can be determined by testing DNA from any tissue of the person's body. Most simply, blood can be drawn and DNA extracted from the cells of the blood. In addition, prenatal diagnosis can be accomplished by testing fetal cells, placental cells or amniotic cells for mutations of the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* gene. Alteration of a wild-type *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* allele, whether, for example, by point mutation or deletion, can be detected by any of the means discussed herein.

There are several methods that can be used to detect DNA sequence variation. Direct DNA sequencing, either manual sequencing or automated fluorescent sequencing can detect sequence variation. Another approach is the single-stranded conformation polymorphism assay (SSCP) (Orita et al., 1989). This method does not detect all sequence changes, especially if the DNA fragment size is greater than 200 bp, but can be optimized to detect most DNA sequence variation. The reduced detection sensitivity is a disadvantage, but the increased throughput possible with SSCP makes it an attractive, viable alternative to direct sequencing for mutation detection on a research basis. The fragments which have shifted mobility on SSCP gels are then sequenced to determine the exact nature of the DNA sequence variation. Other approaches based on the detection of mismatches between the two complementary DNA strands include clamped denaturing gel electrophoresis (CDGE) (Sheffield et al., 1991), heteroduplex analysis (HA) (White et al., 1992) and chemical mismatch cleavage (CMC) (Grompe et al., 1989). None of the methods described above will detect large deletions, duplications or insertions, nor will they detect a regulatory mutation which affects transcription or translation of the protein. Other methods which might detect these classes of mutations such as a protein truncation assay or the asymmetric assay, detect only specific types of mutations and would not detect missense mutations. A review of currently available methods of detecting DNA sequence variation can be found in a recent review by Grompe (1993). Once a mutation is known, an allele specific detection approach such as allele specific oligonucleotide (ASO) hybridization can be utilized to rapidly screen large numbers of other samples for that same mutation. Such a technique can

utilize probes which are labeled with gold nanoparticles to yield a visual color result (Elghanian et al., 1997).

A rapid preliminary analysis to detect polymorphisms in DNA sequences can be performed by looking at a series of Southern blots of DNA cut with one or more restriction enzymes, preferably with a large number of restriction enzymes. Each blot contains a series of normal individuals and a series of LQTS cases. Southern blots displaying hybridizing fragments (differing in length from control DNA when probed with sequences near or including the *HERG* locus) indicate a possible mutation. If restriction enzymes which produce very large restriction fragments are used, then pulsed field gel electrophoresis (PFGE) is employed.

Detection of point mutations may be accomplished by molecular cloning of the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* alleles and sequencing the alleles using techniques well known in the art. Also, the gene or portions of the gene may be amplified, e.g., by PCR or other amplification technique, and the amplified gene or amplified portions of the gene may be sequenced.

There are six well known methods for a more complete, yet still indirect, test for confirming the presence of a susceptibility allele: 1) single stranded conformation analysis (SSCP) (Orita et al., 1989); 2) denaturing gradient gel electrophoresis (DGGE) (Wartell et al., 1990; Sheffield et al., 1989); 3) RNase protection assays (Finkelstein et al., 1990; Kinszler et al., 1991); 4) allele-specific oligonucleotides (ASOs) (Conner et al., 1983); 5) the use of proteins which recognize nucleotide mismatches, such as the *E. coli* mutS protein (Modrich, 1991); and 6) allele-specific PCR (Ruano and Kidd, 1989). For allele-specific PCR, primers are used which hybridize at their 3' ends to a particular *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* mutation. If the particular mutation is not present, an amplification product is not observed. Amplification Refractory Mutation System (ARMS) can also be used, as disclosed in European Patent Application Publication No. 0332435 and in Newton et al., 1989. Insertions and deletions of genes can also be detected by cloning, sequencing and amplification. In addition, restriction fragment length polymorphism (RFLP) probes for the gene or surrounding marker genes can be used to score alteration of an allele or an insertion in a polymorphic fragment. Such a method is particularly useful for screening relatives of an affected individual for the presence of the mutation found in that individual. Other techniques for detecting insertions and deletions as known in the art can be used.

In the first three methods (SSCP, DGGE and RNase protection assay), a new electrophoretic band appears. SSCP detects a band which migrates differentially because the sequence change causes a difference in single-strand, intramolecular base pairing. RNase protection involves cleavage of the mutant polynucleotide into two or more smaller fragments. DGGE detects differences in migration rates of mutant sequences compared to wild-type sequences, using a denaturing gradient gel. In an allele-specific oligonucleotide assay, an oligonucleotide is designed which detects a specific sequence, and the assay is performed by detecting the presence or absence of a hybridization signal. In the mutS assay, the protein binds only to sequences that contain a nucleotide mismatch in a heteroduplex between mutant and wild-type sequences.

Mismatches, according to the present invention, are hybridized nucleic acid duplexes in which the two strands are not 100% complementary. Lack of total homology may be due to deletions, insertions, inversions or substitutions. Mismatch detection can be used to detect point mutations in the gene or in its mRNA product. While these techniques are less sensitive than sequencing, they are simpler to perform on a large number of samples. An example of a mismatch cleavage technique is the RNase protection method. In the practice of the present invention, the method involves the use of a labeled riboprobe which is complementary to the human wild-type *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* gene coding sequence. The riboprobe and either mRNA or DNA isolated from the person are annealed (hybridized) together and subsequently digested with the enzyme RNase A which is able to detect some mismatches in a duplex RNA structure. If a mismatch is detected by RNase A, it cleaves at the site of the mismatch. Thus, when the annealed RNA preparation is separated on an electrophoretic gel matrix, if a mismatch has been detected and cleaved by RNase A, an RNA product will be seen which is smaller than the full length duplex RNA for the riboprobe and the mRNA or DNA. The riboprobe need not be the full length of the mRNA or gene but can be a segment of either. If the riboprobe comprises only a segment of the mRNA or gene, it will be desirable to use a number of these probes to screen the whole mRNA sequence for mismatches.

In similar fashion, DNA probes can be used to detect mismatches, through enzymatic or chemical cleavage. See, e.g., Cotton et al., 1988; Shenk et al., 1975; Novack et al., 1986. Alternatively, mismatches can be detected by shifts in the electrophoretic mobility of mismatched duplexes relative to matched duplexes. See, e.g., Cariello, 1988. With either riboprobes or DNA

probes, the cellular mRNA or DNA which might contain a mutation can be amplified using PCR (see below) before hybridization. Changes in DNA of the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* gene can also be detected using Southern hybridization, especially if the changes are gross rearrangements, such as deletions and insertions.

DNA sequences of the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* gene which have been amplified by use of PCR may also be screened using allele-specific probes. These probes are nucleic acid oligomers, each of which contains a region of the gene sequence harboring a known mutation. For example, one oligomer may be about 30 nucleotides in length, corresponding to a portion of the gene sequence. By use of a battery of such allele-specific probes, PCR amplification products can be screened to identify the presence of a previously identified mutation in the gene. Hybridization of allele-specific probes with amplified *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* sequences can be performed, for example, on a nylon filter. Hybridization to a particular probe under high stringency hybridization conditions indicates the presence of the same mutation in the tissue as in the allele-specific probe.

The newly developed technique of nucleic acid analysis via microchip technology is also applicable to the present invention. In this technique, literally thousands of distinct oligonucleotide probes are built up in an array on a silicon chip. Nucleic acid to be analyzed is fluorescently labeled and hybridized to the probes on the chip. It is also possible to study nucleic acid-protein interactions using these nucleic acid microchips. Using this technique one can determine the presence of mutations or even sequence the nucleic acid being analyzed or one can measure expression levels of a gene of interest. The method is one of parallel processing of many, even thousands, of probes at once and can tremendously increase the rate of analysis. Several papers have been published which use this technique. Some of these are Hacia et al., 1996; Shoemaker et al., 1996; Chee et al., 1996; Lockhart et al., 1996; DeRisi et al., 1996; Lipshutz et al., 1995. This method has already been used to screen people for mutations in the breast cancer gene *BRCA1* (Hacia et al., 1996). This new technology has been reviewed in a news article in Chemical and Engineering News (Borman, 1996) and been the subject of an editorial (Editorial, Nature Genetics, 1996). Also see Fodor (1997).

The most definitive test for mutations in a candidate locus is to directly compare genomic *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* sequences from patients with those from a control

population. Alternatively, one could sequence messenger RNA after amplification, e.g., by PCR, thereby eliminating the necessity of determining the exon structure of the candidate gene.

Mutations from patients falling outside the coding region of *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* can be detected by examining the non-coding regions, such as introns and regulatory sequences near or within the genes. An early indication that mutations in noncoding regions are important may come from Northern blot experiments that reveal messenger RNA molecules of abnormal size or abundance in patients as compared to control individuals.

Alteration of *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* mRNA expression can be detected by any techniques known in the art. These include Northern blot analysis, PCR amplification and RNase protection. Diminished mRNA expression indicates an alteration of the wild-type gene. Alteration of wild-type genes can also be detected by screening for alteration of wild-type KVLQT1, HERG, SCN5A, KCNE1 or KCNE2 protein. For example, monoclonal antibodies immunoreactive with HERG can be used to screen a tissue. Lack of cognate antigen would indicate a mutation. Antibodies specific for products of mutant alleles could also be used to detect mutant gene product. Such immunological assays can be done in any convenient formats known in the art. These include Western blots, immunohistochemical assays and ELISA assays. Any means for detecting an altered KVLQT1, HERG, SCN5A, KCNE1 or KCNE2 protein can be used to detect alteration of wild-type *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* genes. Functional assays, such as protein binding determinations, can be used. In addition, assays can be used which detect KVLQT1, HERG, SCN5A, KCNE1 or KCNE2 biochemical function. Finding a mutant *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* gene product indicates alteration of a wild-type *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* gene.

Mutant *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* genes or gene products can also be detected in other human body samples, such as serum, stool, urine and sputum. The same techniques discussed above for detection of mutant genes or gene products in tissues can be applied to other body samples. By screening such body samples, a simple early diagnosis can be achieved for hereditary LQTS.

Initially, the screening method involves amplification of the relevant *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* sequence. In another preferred embodiment of the invention, the screening method involves a non-PCR based strategy. Such screening methods include two-step label amplification methodologies that are well known in the art. Both PCR and non-PCR based

screening strategies can detect target sequences with a high level of sensitivity. Further details of these methods are briefly presented below and further descriptions can be found in PCT published application WO 96/05306, incorporated herein by reference.

The most popular method used today is target amplification. Here, the target nucleic acid sequence is amplified with polymerases. One particularly preferred method using polymerase-driven amplification is the polymerase chain reaction (PCR). The polymerase chain reaction and other polymerase-driven amplification assays can achieve over a million-fold increase in copy number through the use of polymerase-driven amplification cycles. Once amplified, the resulting nucleic acid can be sequenced or used as a substrate for DNA probes.

When the probes are used to detect the presence of the target sequences, the biological sample to be analyzed, such as blood or serum, may be treated, if desired, to extract the nucleic acids. The sample nucleic acid may be prepared in various ways to facilitate detection of the target sequence; e.g. denaturation, restriction digestion, electrophoresis or dot blotting. The targeted region of the analyte nucleic acid usually must be at least partially single-stranded to form hybrids with the targeting sequence of the probe. If the sequence is naturally single-stranded, denaturation will not be required. However, if the sequence is double-stranded, the sequence will probably need to be denatured. Denaturation can be carried out by various techniques known in the art.

Analyte nucleic acid and probe are incubated under conditions which promote stable hybrid formation of the target sequence in the probe with the putative targeted sequence in the analyte. The region of the probes which is used to bind to the analyte can be made completely complementary to the targeted region of the genes. Therefore, high stringency conditions are desirable in order to prevent false positives. However, conditions of high stringency are used only if the probes are complementary to regions of the chromosome which are unique in the genome. The stringency of hybridization is determined by a number of factors during hybridization and during the washing procedure, including temperature, ionic strength, base composition, probe length, and concentration of formamide. Under certain circumstances, the formation of higher order hybrids, such as triplexes, quadraplexes, etc., may be desired to provide the means of detecting target sequences.

Detection, if any, of the resulting hybrid is usually accomplished by the use of labeled probes. Alternatively, the probe may be unlabeled, but may be detectable by specific binding

with a ligand which is labeled, either directly or indirectly. Suitable labels, and methods for labeling probes and ligands are known in the art, and include, for example, radioactive labels which may be incorporated by known methods (e.g., nick translation, random priming or kinasing), biotin, fluorescent groups, chemiluminescent groups (e.g., dioxetanes, particularly triggered dioxetanes), enzymes, antibodies and the like. Variations of this basic scheme are known in the art, and include those variations that facilitate separation of the hybrids to be detected from extraneous materials and/or that amplify the signal from the labeled moiety. A number of these variations are well known.

As noted above, non-PCR based screening assays are also contemplated in this invention. This procedure hybridizes a nucleic acid probe (or an analog such as a methyl phosphonate backbone replacing the normal phosphodiester), to the low level DNA target. This probe may have an enzyme covalently linked to the probe, such that the covalent linkage does not interfere with the specificity of the hybridization. This enzyme-probe-conjugate-target nucleic acid complex can then be isolated away from the free probe enzyme conjugate and a substrate is added for enzyme detection. Enzymatic activity is observed as a change in color development or luminescent output resulting in a 10^3-10^6 increase in sensitivity. For example, the preparation of oligodeoxynucleotide-alkaline phosphatase conjugates and their use as hybridization probes are well known.

Two-step label amplification methodologies are known in the art. These assays work on the principle that a small ligand (such as digoxigenin, biotin, or the like) is attached to a nucleic acid probe capable of specifically binding the target gene. Allele specific probes are also contemplated within the scope of this example.

In one example, the small ligand attached to the nucleic acid probe is specifically recognized by an antibody-enzyme conjugate. In one embodiment of this example, digoxigenin is attached to the nucleic acid probe. Hybridization is detected by an antibody-alkaline phosphatase conjugate which turns over a chemiluminescent substrate. In a second example, the small ligand is recognized by a second ligand-enzyme conjugate that is capable of specifically complexing to the first ligand. A well known embodiment of this example is the biotin-avidin type of interactions. Methods for labeling nucleic acid probes and their use in biotin-avidin based assays are well known.

It is also contemplated within the scope of this invention that the nucleic acid probe assays of this invention will employ a cocktail of nucleic acid probes capable of detecting the gene or genes. Thus, in one example to detect the presence of *KVLQT1* in a cell sample, more than one probe complementary to *KVLQT1* is employed and in particular the number of different probes is alternatively 2, 3, or 5 different nucleic acid probe sequences. In another example, to detect the presence of mutations in the *KVLQT1* gene sequence in a patient, more than one probe complementary to *KVLQT1* is employed where the cocktail includes probes capable of binding to the allele-specific mutations identified in populations of patients with alterations in *KVLQT1*. In this embodiment, any number of probes can be used.

Large amounts of the polynucleotides of the present invention may be produced by replication in a suitable host cell. Natural or synthetic polynucleotide fragments coding for a desired fragment will be incorporated into recombinant polynucleotide constructs, usually DNA constructs, capable of introduction into and replication in a prokaryotic or eukaryotic cell. Usually the polynucleotide constructs will be suitable for replication in a unicellular host, such as yeast or bacteria, but may also be intended for introduction to (with and without integration within the genome) cultured mammalian or plant or other eukaryotic cell lines. The purification of nucleic acids produced by the methods of the present invention are described, e.g., in Sambrook et al., 1989 or Ausubel et al., 1992.

The polynucleotides of the present invention may also be produced by chemical synthesis, e.g., by the phosphoramidite method described by Beaucage and Caruthers (1981) or the triester method according to Matteucci and Caruthers (1981) and may be performed on commercial, automated oligonucleotide synthesizers. A double-stranded fragment may be obtained from the single-stranded product of chemical synthesis either by synthesizing the complementary strand and annealing the strand together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

Polynucleotide constructs prepared for introduction into a prokaryotic or eukaryotic host may comprise a replication system recognized by the host, including the intended polynucleotide fragment encoding the desired polypeptide, and will preferably also include transcription and translational initiation regulatory sequences operably linked to the polypeptide encoding segment. Expression vectors may include, for example, an origin of replication or autonomously replicating sequence (ARS) and expression control sequences, a promoter, an enhancer and

necessary processing information sites, such as ribosome-binding sites, RNA splice sites, polyadenylation sites, transcriptional terminator sequences, and mRNA stabilizing sequences. Such vectors may be prepared by means of standard recombinant techniques well known in the art and discussed, for example, in Sambrook et al. (1989) or Ausubel et al. (1992).

An appropriate promoter and other necessary vector sequences will be selected so as to be functional in the host, and may include, when appropriate, those naturally associated with the *KVLQT1* or other gene. Examples of workable combinations of cell lines and expression vectors are described in Sambrook et al. (1989) or Ausubel et al. (1992); see also, e.g., Metzger et al. (1988). Many useful vectors are known in the art and may be obtained from such vendors as Stratagene, New England Biolabs, Promega Biotech, and others. Promoters such as the trp, lac and phage promoters, tRNA promoters and glycolytic enzyme promoters may be used in prokaryotic hosts. Useful yeast promoters include promoter regions for metallothionein, 3-phosphoglycerate kinase or other glycolytic enzymes such as enolase or glyceraldehyde-3-phosphate dehydrogenase, enzymes responsible for maltose and galactose utilization, and others. Vectors and promoters suitable for use in yeast expression are further described in Hitzeman et al., EP 73,675A. Appropriate non-native mammalian promoters might include the early and late promoters from SV40 (Fiers et al., 1978) or promoters derived from murine Molony leukemia virus, mouse tumor virus, avian sarcoma viruses, adenovirus II, bovine papilloma virus or polyoma. Insect promoters may be derived from baculovirus. In addition, the construct may be joined to an amplifiable gene (e.g., DHFR) so that multiple copies of the gene may be made. For appropriate enhancer and other expression control sequences, see also Enhancers and Eukaryotic Gene Expression, Cold Spring Harbor Press, Cold Spring Harbor, New York (1983). See also, e.g., U.S. Patent Nos. 5,691,198; 5,735,500; 5,747,469 and 5,436,146.

While such expression vectors may replicate autonomously, they may also replicate by being inserted into the genome of the host cell, by methods well known in the art.

Expression and cloning vectors will likely contain a selectable marker, a gene encoding a protein necessary for survival or growth of a host cell transformed with the vector. The presence of this gene ensures growth of only those host cells which express the inserts. Typical selection genes encode proteins that a) confer resistance to antibiotics or other toxic substances, e.g. ampicillin, neomycin, methotrexate, etc., b) complement auxotrophic deficiencies, or c) supply critical nutrients not available from complex media, e.g., the gene encoding D-alanine

racemase for *Bacilli*. The choice of the proper selectable marker will depend on the host cell, and appropriate markers for different hosts are well known in the art.

The vectors containing the nucleic acids of interest can be transcribed *in vitro*, and the resulting RNA introduced into the host cell by well-known methods, e.g., by injection (see, Kubo et al. (1988)), or the vectors can be introduced directly into host cells by methods well known in the art, which vary depending on the type of cellular host, including electroporation; transfection employing calcium chloride, rubidium chloride calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; infection (where the vector is an infectious agent, such as a retroviral genome); and other methods. See generally, Sambrook et al. (1989) and Ausubel et al. (1992). The introduction of the polynucleotides into the host cell by any method known in the art, including, *inter alia*, those described above, will be referred to herein as "transformation." The cells into which have been introduced nucleic acids described above are meant to also include the progeny of such cells.

Large quantities of the nucleic acids and polypeptides of the present invention may be prepared by expressing the *KVLQT1* nucleic acid or portions thereof in vectors or other expression vehicles in compatible prokaryotic or eukaryotic host cells. The most commonly used prokaryotic hosts are strains of *Escherichia coli*, although other prokaryotes, such as *Bacillus subtilis* or *Pseudomonas* may also be used.

Mammalian or other eukaryotic host cells, such as those of yeast, filamentous fungi, plant, insect, or amphibian or avian species, may also be useful for production of the proteins of the present invention. Propagation of mammalian cells in culture is *per se* well known. See, Jakoby and Pastan (eds.) (1979). Examples of commonly used mammalian host cell lines are VERO and HeLa cells, Chinese hamster ovary (CHO) cells, and WI38, BHK, and COS cell lines, although it will be appreciated by the skilled practitioner that other cell lines may be appropriate, e.g., to provide higher expression, desirable glycosylation patterns, or other features. An example of a commonly used insect cell line is SF9.

Clones are selected by using markers depending on the mode of the vector construction. The marker may be on the same or a different DNA molecule, preferably the same DNA molecule. In prokaryotic hosts, the transformant may be selected, e.g., by resistance to ampicillin, tetracycline or other antibiotics. Production of a particular product based on temperature sensitivity may also serve as an appropriate marker.

Prokaryotic or eukaryotic cells transformed with the polynucleotides of the present invention will be useful not only for the production of the nucleic acids and polypeptides of the present invention, but also, for example, in studying the characteristics of KVLQT1 or other polypeptides.

The probes and primers based on the *KVLQT1* or other gene sequences disclosed herein are used to identify homologous *KVLQT1* or other gene sequences and proteins in other species. These gene sequences and proteins are used in the diagnostic/prognostic, therapeutic and drug screening methods described herein for the species from which they have been isolated.

The studies described in the Examples below resulted in the determination of many novel mutations. Previous studies had defined 126 distinct disease causing mutations in the LQTS genes *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* and *KCNE2* (Wang Q. et al., 1996a; Curran et al., 1995; Wang et al., 1995a; Splawski et al., 1997a; Abbott et al., 1999; Chouabe et al., 1997; Wollnik et al., 1997; Neyroud et al., 1997; Splawski et al., 1997b; Tyson et al., 1997; Schulze-Bahr et al., 1997; Priori et al., 1999; Splawski et al., 1998; Wang et al., 1995b; Russell et al., 1996; Neyroud et al., 1998; Neyroud et al., 1999; Donger et al., 1997; Tanaka et al., 1997; Jongbloed et al., 1999; Priori et al., 1998; Itoh et al., 1998a; Itoh et al., 1998b; Mohammad-Panah et al., 1999; Saarinen et al., 1998; Ackerman et al., 1998; Berthet et al., 1999; Kanters, 1998; van den Berg et al., 1997; Dausse et al., 1996; Benson et al., 1996; Akimoto et al., 1998; Satler et al., 1996; Satler et al., 1998; Makita et al., 1998, An et al., 1998; Schulze-Bahr et al., 1995; Duggal et al., 1998; Chen Q. et al., 1999; Li et al., 1998; Wei et al., 1999; Larsen et al., 1999a; Bianchi et al., 1999; Ackerman et al., 1999a; Ackerman et al., 1999b; Murray et al., 1999; Larsen et al., 1999b; Yoshida et al., 1999; Wattanasirichaigoon et al., 1999; Bezzina et al., 1999; Hoorntje et al., 1999). The sequence of each wild-type gene has been published. The *KVLQT1* can be found in Splawski et al. (1998) and the coding region of the cDNA is shown herein as SEQ ID NO:1 and the encoded KVLQT1 is shown as SEQ ID NO:2. SCN5A was reported by Gellens et al. (1992) and its sequence is provided by GenBank Accession No. NM_000335. The coding sequence of *SCN5A* is shown herein as SEQ ID NO:3 and the encoded SCN5A is shown as SEQ ID NO:4. Most of the mutations were found in *KVLQT1* (Yoshida et al., 1999) and *HERG* (Itoh et al., 1998b), and fewer in *SCN5A* (Wang Q. et al., 1996a), *KCNE1* (Jiang et al., 1994) and *KCNE2* (Ward, 1964). These mutations were identified in regions with known intron/exon structure, primarily the transmembrane and pore domains. In this study, we screened 262 individuals with

LQTS for mutations in all known arrhythmia genes. We identified 134 mutations, 80 of which were novel. Together with 43 mutations reported in our previous studies, we have now identified 177 mutations in these 262 LQTS individuals (68%). The failure to identify mutations in 32% of the individuals may result from phenotypic errors, incomplete sensitivity of SSCP or presence of mutations in regulatory sequences. However, it is also clear that additional LQTS genes await discovery (Jiang et al., 1994; Schott et al., 1995).

Missense mutations were most common (72%), followed by frameshift mutations (10%), in-frame deletions, nonsense and splice site mutations (5-7% each). Most mutations resided in intracellular (52%) and transmembrane (30%) domains; 12% were found in pore and 6% in extracellular segments. One hundred one of the 129 distinct LQTS mutations (78%) were identified in single families or individuals. Most of the 177 mutations were found in *KVLQT1* (75 or 42%) and *HERG* (80 or 45%). These two genes accounted for 87% of the identified mutations, while mutations in *SCN5A* (14 or 8%), *KCNE1* (5 or 3%) and *KCNE2* (3 or 2%) accounted for the other 13%.

Multiple mutations were found in regions encoding S5, S5/P, P and S6 of *KVLQT1* and *HERG*. The P region of potassium channels forms the outer pore and contains the selectivity filter (Doyle et al., 1998). Transmembrane segment 6, corresponding to the inner helix of KcsA, forms the inner 2/3 of the pore. This structure is supported by the S5 transmembrane segment, corresponding to the outer helix of KcsA, and is conserved from prokaryotes to eukaryotes ((MacKinnon et al., 1998). Mutations in these regions will likely disrupt potassium transport. Many mutations were identified in the C-termini of *KVLQT1* and *HERG*. Changes in the C-terminus of HERG could lead to anomalies in tetramerization as it has been proposed that the C-terminus of eag, which is related to HERG, is involved in this process (Ludwig et al, 1994).

Multiple mutations were also identified in regions that were different for *KVLQT1* and *HERG*. In *KVLQT1*, multiple mutations were found in the sequences coding for the S2/S3 and S4/S5 linkers. Coexpression of S2/S3 mutants with wild-type KVLQT1 in *Xenopus* oocytes led to simple loss of function or dominant-negative effect without significantly changing the biophysical properties of I_{Ks} channels (Chouabe et al., 1997; Shalaby et al., 1997; Wang et al., 1999). On the other hand, S4/S5 mutations altered the gating properties of the channels and modified KVLQT1 interactions with minK subunits (Wang et al., 1999; Franqueza et al., 1999).

In *HERG*, more than 20 mutations were identified in the N-terminus. HERG channels lacking this region deactivate faster and mutations in the region had a similar effect (Chen J. et al., 1999).

Mutations in *KCNE1* and *KCNE2*, encoding minK and MiRP1, the respective I_{Ks} and I_{Kr} β-subunits, altered the biophysical properties of the channels (Splawski et al., 1997a; Abbott et al., 1999; Sesti and Goldstein, 1998). A MiRP1 mutant, involved in clarithromyocin-induced arrhythmia, increased channel blockade by the antibiotic (Abbott et al., 1999). Mutations in *SCN5A*, the sodium channel α-subunit responsible for cardiac I_{Na}, destabilized the inactivation gate causing delayed channel inactivation and dispersed reopenings (Bennett et al., 1995; Dumaine et al., 1996; Wei et al., 1999; Wang DW et al., 1996). One SCN5A mutant affected the interactions with the sodium channel β-subunit (An et al., 1998).

It is interesting to note that probands with *KCNE1* and *KCNE2* mutations were older and had shorter QTc than probands with the other genotypes. The significance of these differences is unknown, however, as the number of probands with *KCNE1* and *KCNE2* genotypes was small.

This catalogue of mutations will facilitate genotype-phenotype analyses. It also has clinical implications for presymptomatic diagnosis and, in some cases, for therapy. Patients with mutations in *KVLQT1*, *HERG*, *KCNE1* and *KCNE2*, for example, may benefit from potassium therapy (Compton et al., 1996). Sodium channel blockers, on the other hand, might be helpful in patients with *SCN5A* mutations (Schwartz et al. (1995). The identification of mutations is of importance for ion channel studies as well. The expression of mutant channels in heterologous systems can reveal how structural changes influence the behavior of the channel or how mutations affect processing (Zhou et al., 1998; Furutani et al., 1999). These studies improve our understanding of channel function and provide insights into mechanisms of disease. Finally, mutation identification will contribute to the development of genetic screening for arrhythmia susceptibility.

The present invention is described by reference to the following Examples, which are offered by way of illustration and are not intended to limit the invention in any manner. Standard techniques well known in the art or the techniques specifically described in the Examples were utilized.

Example 1

Ascertainment and Phenotyping

Individuals were ascertained in clinics from North America and Europe. Individuals were evaluated for LQTS based on QTc (the QT interval corrected for heart rate) and for the presence of symptoms. In this study, we focused on the probands. Individuals show prolongation of the QT interval (QTc≥460 ms) and/or documented *torsade de pointes*, ventricular fibrillation, cardiac arrest or aborted sudden death. Informed consent was obtained in accordance with local institutional review board guidelines. Phenotypic data were interpreted without knowledge of genotype. Sequence changes altering coding regions or predicted to affect splicing that were not detected in at least 400 control chromosomes were defined as mutations. No changes except known polymorphisms were detected ina ny of the genes in the control population. This does not exclude the possibility that some mutations are rare variants not associated with disease.

Example 2

Mutational Analyses

To determine the spectrum of LQTS mutations, we used SSCP (Single Stand Conformation Polymorphism) and DNA sequence analyses to screen 262 unrelated individuals with LQTS. Seventeen primer pairs were used to screen *KVLQT1* (Splawski et al., 1998), twenty-one primer pairs were used for *HERG* (Splawski et al., 1998) and three primer pairs were used for *KCNE1* (Splawski et al., 1997a) and *KCNE2* (Abbott et al., 1999). Thirty-three primer pairs (Wang Q. et al., 1996b) were used in SSCP analysis to screen all *SCN5A* exons in 50 individuals with suspected abnormalities in I_{Na}. Exons 23-28, in which mutations were previously identified, were screened in all 262 individuals.

Gender, age, QTc and presence of symptoms are summarized in Table 1. The average age at ascertainment was 29 with a corrected QT interval of 492 ms. Seventy-five percent had a history of symptoms and females predominated with an ~ 2:1 ratio. Although the numbers were small, corrected QT intervals for individuals harboring *KCNE1* and *KCNE2* mutations were shorter at 457 ms.

Table 1

Age, QTc, Gender and Presence of Symptoms

Genotype	Age*, y (mean±SD)	Gender (F/M)	QTc, ms (mean±SD)	Symptoms†
KVLQT1	32 ± 19	52/23	493 ± 45	78%
HERG	31 ± 19	51/29	498 ± 48	71%
SCN5A	32 ± 24	8/6	511 ± 42	55%
KCNE1	43 ± 16	3/2	457 ± 25	40%
KCNE2	54 ± 20	3/0	457 ± 05	67%
unknown	25 ± 16	56/29	484 ± 46	81%
all	29 ± 19	173/89	492 ± 47	75%

* - age at ascertainment

† - symptoms include syncope, cardiac arrest or sudden death

The SSCP analyses revealed many mutations. *KVLQT1* mutations associated with LQTS were identified in 52 individuals (Figure 1 and Table 2). Twenty of the mutations were novel. *HERG* mutations were identified in 68 LQTS individuals (Figure 2 and Table 3). Fifty-two of these mutations were novel. *SCN5A* mutations were identified in eight cases (Figure 3 and Table 4). Five of the mutations were novel. Three novel *KCNE1* mutations were identified (Figure 4 and Table 5) and three mutations were identified in *KCNE2* (Figure 5 and Table 6) (Abbott et al., 1999). None of the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* and *KCNE2* mutations was observed in 400 control chromosomes.

Table 2

Summary of All *KVLQT1* Mutations*

Nucleotide Change†	Coding Effect	Position	Exon	Number of families‡	Study
del211-219	del71-73	N-terminus	1	1	Ackerman et al., 1999a
A332G †	Y111C	N-terminus	1	1	This

Nucleotide Change†	Coding Effect	Position	Exon	Number of families‡	Study
del451-452	A150fs/132	S2	2	1 JLN	Chen Q. et al., 1999
T470G	F157C	S2	1	1	Larsen et al., 1999a
G477+1A	M159sp	S2	2	1 JLN, 1 UK	This; Donger et al., 1997
G477+5A	M159sp	S2	1	1	Ackerman et al., 1999b
G478A †	E160K	S2	3	1	This
del500-502	F167W/del G168	S2	3	1	Wang Q. et al., 1996a
G502A	G168R	S2	3	7	This; Splawski et al., 1998; Donger et al., 1997
C520T	R174C	S2/S3	3	1	Donger et al., 1997
G521A †	R174H	S2/S3	3	1	This
G532A	A178T	S2/S3	3	1	Tanaka et al., 1997
G532C	A178P	S2/S3	3	1	Wang Q. et al., 1996a
G535A †	G179S	S2/S3	3	1	This
A551C	Y184S	S2/S3	3	2	This; Jongbloed et al., 1999
G565A	G189R	S2/S3	3	3	Wang Q. et al., 1996a; Jongbloed et al., 1999
insG567-568	G189fs/94	S2/S3	3	1 (RW + JLN)	Splawski et al., 1997b
G569A	R190Q	S2/S3	3	2	Splawski et al., 1998; Donger et al., 1997
del572-576	L191fs/90	S2/S3	3	1 JLN, 1 RW 2 (JLN + RW)	Tyson et al., 1997; Ackerman et al., 1999b

Nucleotide Change†	Coding Effect	Position	Exon	Number of families‡	Study
G580C †	A194P	S2/S3	3	1	This
C674T	S225L	S4	4	2	This; Priori et al., 1999
G724A	D242N	S4/S5	5	1	Itoh et al., 1998b
C727T †	R243C	S4/S5	5	2	This
G728A	R243H	S4/S5	5	1 JLN	Saarinen et al., 1998
T742C †	W248R	S4/S5	5	1	This
T749A	L250H	S4/S5	5	1	Itoh et al., 1998a
G760A	V254M	S4/S5	5	4	This; Wang Q. et al., 1996a; Donger et al., 1997
G781A	E261K	S4/S5	6	1	Donger et al., 1997
T797C †	L266P	S5	6	1	This
G805A	G269S	S5	6	1	Ackerman et al., 1999b
G806A	G269D	S5	6	3	This; Donger et al., 1997
C817T	L273F	S5	6	2	This; Wang Q. et al., 1996a
A842G	Y281C	S5	6	1	Priori et al., 1999
G898A	A300T	S5/Pore	6	1	Priori et al., 1998
G914C	W305S	Pore	6	1 JLN	Chouabe et al., 1997
G916A	G306R	Pore	6	1	Wang Q. et al, 1996a
del921-(921+2)	V307sp	Pore	6	1	Li et al., 1998
G921+1T †	V307sp	Pore	6	1	This
A922-2C †	V307sp	Pore	7	1	This
G922-1C	V307sp	Pore	7	1	Murray et al., 1999
C926G	T309R	Pore	7	1	Donger et al., 1997

Nucleotide Change†	Coding Effect	Position	Exon	Number of families‡	Study
G928A †	V310I	Pore	7	1	This
C932T	T311I	Pore	7	1	Saarinen et al., 1998
C935T	T312I	Pore	7	2	This; Wang Q. et al., 1996a
C939G	I313M	Pore	7	1	Tanaka et al., 1997
G940A	G314S	Pore	7	7	Splawski et al., 1998; Russell et al., 1996; Donger et al., 1997; Jongbloed et al., 1999; Itoh et al., 1998b
A944C	Y315S	Pore	7	3	Donger et al., 1997; Jongbloed et al., 1999
A944G	Y315C	Pore	7	2	Priori et al., 1999; Splawski et al., 1998
G949A	D317N	Pore	7	2	Wollnik et al., 1997; Saarinen et al., 1998
G954C	K318N	Pore	7	1	Splawski et al., 1998
C958G	P320A	Pore	7	1	Donger et al., 1997
G973A	G325R	S6	7	4	This; Donger et al., 1997; Tanaka et al., 1997
del1017-1019	delF340	S6	7	2	This; Ackerman et al., 1998
C1022A	A341E	S6	7	5	This; Wang Q. et al., 1996a; Berthet et al., 1999

Nucleotide Change†	Coding Effect	Position	Exon	Number of families‡	Study
C1022T	A341V	S6	7	7	This; Wang Q. et al., 1996a; Russell et al., 1996; Donger et al., 1997; Li et al., 1998
C1024T	L342F	S6	7	1	Donger et al., 1997
C1031T	A344V	S6	7	1	Donger et al., 1997
G1032A	A344sp	S6	7	9	This; Kanters, 1998; Li et al., 1998; Ackerman et al., 1999b; Murray et al., 1999
G1032C	A344sp	S6	7	1	Murray et al., 1999
G1033C	G345R	S6	8	1	van den Berg et al., 1997
G1034A	G345E	S6	8	1	Wang Q. et al., 1996a
C1046G †	S349W	S6	8	1	This
T1058C	L353P	S6	8	1	Splawski et al., 1998
C1066T †	Q356X	C-terminus	8	1	This
C1096T	R366W	C-terminus	8	1	Splawski et al., 1998
G1097A †	R366Q	C-terminus	8	1	This
G1097C	R366P	C-terminus	8	1	Tanaka et al., 1997
G1111A	A371T	C-terminus	8	1	Donger et al., 1997
T1117C	S373P	C-terminus	8	1	Jongbloed et al., 1999
C1172T †	T391I	C-terminus	9	1	This
T1174C	W392R	C-terminus	9	1	Jongbloed et al., 1999
C1343G †	P448R	C-terminus	10	2	This
C1522T	R518X	C-terminus	12	1 JLN, 3 RW	This; Larsen et al., 1999

Nucleotide Change†	Coding Effect	Position	Exon	Number of families‡	Study
G1573A	A525T	C-terminus	12	1	Larsen et al., 1999b
C1588T †	Q530X	C-terminus	12	1 JLN, 1 RW	This
C1615T	R539W	C-terminus	13	1	Chouabe et al., 1997
del6/ins7	E543fs/107	C-terminus	13	1 JLN	Neyroud et al., 1997
C1663T	R555C	C-terminus	13	3	Donger et al., 1997
C1697T †	S566F	C-terminus	14	3	This
C1747T †	R583C	C-terminus	15	1	This
C1760T	T587M	C-terminus	15	1 JLN, 1 RW	Donger et al., 1997; Itoh et al., 1998b
G1772A	R591H	C-terminus	15	1	Donger et al., 1997
G1781A †	R594Q	C-terminus	15	3	This
del1892-1911	P630fs/13	C-terminus	16	1 JLN	Donger et al., 1997
insC1893-1894	P631fs/19	C-terminus	16	1	Donger et al., 1997

* - ins denotes insertion; del denotes deletion; sp denotes the last unaffected amino acid before the predicted splice mutation; fs denotes the last amino acid unaffected by a frameshift, following fs is the number of amino acids before termination; X denotes a stop codon occurred.

† - denotes novel mutation

‡ - Number of Romano-Ward families unless otherwise indicated (UK - unknown)

Table 3

Summary of All *HERG* Mutations*

Nucleotide Change	Coding Effect	Position	Exon	Number of RW Families	Study
C87A †	F29L	N-terminus	2	1	This
A98C †	N33T	N-terminus	2	2	This
C132A †	C44X	N-terminus	2	1	This
G140T †	G47V	N-terminus	2	1	This
G157C †	G53R	N-terminus	2	1	This
G167A †	R56Q	N-terminus	2	1	This
T196G †	C66G	N-terminus	2	1	This
A209G †	H70R	N-terminus	2	2	This
C215A †	P72Q	N-terminus	2	2	This
del221-251 †	R73fs/31	N-terminus	2	1	This
G232C †	A78P	N-terminus	2	1	This
dupl234-250†	A83fs/37	N-terminus	2	1	This
C241T †	Q81X	N-terminus	2	1	This
T257G †	L86R	N-terminus	2	1	This
insC422-423†	P141fs/2	N-terminus	3	1	This
insC453-454†	P151fs/179	N-terminus	3	1	This
dupl558-600	L200fs/144	N-terminus	4	1	Hoorntje et al., 1999
insC724-725†	P241fs/89	N-terminus	4	1	This
del885 †	V295fs/63	N-terminus	4	1	This
C934T †	R312C	N-terminus	5	1	This
C1039T †	P347S	N-terminus	5	1	This
G1128A †	Q376sp	N-terminus	5	1	This

Nucleotide Change	Coding Effect	Position	Exon	Number of RW Families	Study
A1129-2G †	Q376sp	N-terminus	6	1	This
del1261	Y420fs/12	S1	6	1	Curran et al., 1995
C1283A	S428X	S1/S2	6	1	Priori et al., 1999
C1307T	T436M	S1/S2	6	1	Priori et al., 1999
A1408G	N470D	S2	6	1	Curran et al., 1995
C1421T	T474I	S2/S3	6	1	Tanaka et al., 1997
C1479G	Y493X	S2/S3	6	1	Itoh et al., 1998a
del1498-1524	del500-508	S3	6	1	Curran et al., 1995
G1592A †	R531Q	S4	7	1	This
C1600T	R534C	S4	7	1	Itoh et al., 1998a
T1655C †	L552S	S5	7	1	This
delT1671	T556fs/7	S5	7	1	Schulze-Bahr et al., 1995
G1672C	A558P	S5	7	1	Jongbloed et al., 1999
G1681A	A561T	S5	7	4	This; Dausse et al., 1996
C1682T	A561V	S5	7	4	This; Curran et al., 1995; Priori et al., 1999
G1714C	G572R	S5/Pore	7	1	Larsen et al., 1999a
G1714T	G572C	S5/Pore	7	1	Splawski et al., 1998
C1744T	R582C	S5/Pore	7	1	Jongbloed et al., 1999
G1750A †	G584S	S5/Pore	7	1	This
G1755T †	W585C	S5/Pore	7	1	This
A1762G	N588D	S5/Pore	7	1	Splawski et al., 1998
T1778C †	I593T	S5/Pore	7	1	This
T1778G	I593R	S5/Pore	7	1	Benson et al., 1996
G1801A	G601S	S5/Pore	7	1	Akimoto et al., 1998

Nucleotide Change	Coding Effect	Position	Exon	Number of RW Families	Study
G1810A	G604S	S5/Pore	7	2	This; Jongbloed et al., 1999
G1825A †	D609N	S5/Pore	7	1	This
T1831C	Y611H	S5/Pore	7	1	Tanaka et al., 1997
T1833 (A or G)	Y611X	S5/Pore	7	1	Schulze-Bahr et al., 1995
G1834T	V612L	Pore	7	1	Satler et al., 1998
C1838T	T613M	Pore	7	4	This; Jongbloed et al., 1999
C1841T	A614V	Pore	7	6	Priori et al., 1999; Splawski et al., 1998; Tanaka et al., 1997; Satler et al., 1998
C1843G †	L615V	Pore	7	1	This
G1876A †	G626S	Pore	7	1	This
C1881G †	F627L	Pore	7	1	This
G1882A	G628S	Pore	7	2	This; Curran et al., 1995
A1885G	N629D	Pore	7	1	Satler et al., 1998
A1886G	N629S	Pore	7	1	Satler et al., 1998
C1887A	N629K	Pore	7	1	Yoshida et al., 1999
G1888C	V630L	Pore	7	1	Tanaka et al., 1997
T1889C	V630A	Pore	7	1	Splawski et al., 1998
C1894T †	P632S	Pore	7	1	This
A1898G	N633S	Pore	7	1	Satler et al., 1998
A1912G †	K638E	S6	7	1	This
del1913-1915†	delK638	S6	7	1	This

Nucleotide Change	Coding Effect	Position	Exon	Number of RW Families	Study
C1920A	F640L	S6	7	1	Jongbloed et al., 1999
A1933T †	M645L	S6	7	1	This
del1951-1952	L650fs/2	S6	8	1	Itoh et al., 1998a
G2044T †	E682X	S6/cNBD	8	1	This
C2173T	Q725X	S6/cNBD	9	1	Itoh et al., 1998a
insT2218-2219 †	H739fs/63	S6/cNBD	9	1	This
C2254T †	R752W	S6/cNBD	9	1	This
dupl2356-2386	V796fs/22	cNBD	9	1	Itoh et al., 1998a
del2395 †	I798fs/10	cNBD	9	1	This
G2398+1C	L799sp	cNBD	9	2	This; Curran et al., 1995
T2414C †	F805S	cNBD	10	1	This
T2414G †	F805C	cNBD	10	1	This
C2453T	S818L	cNBD	10	1	Berthet et al., 1999
G2464A	V822M	cNBD	10	2	Berthet et al., 1999; Satler et al., 1996
C2467T †	R823W	cNBD	10	2	This
A2582T †	N861I	C-terminus	10	1	This
G2592+1A	D864sp	C-terminus	10	2	This; Berthet et al., 1999
del2660 †	K886fs/85	C-terminus	11	1	This
C2750T †	P917L	C-terminus	12	1	This
del2762 †	R920fs/51	C-terminus	12	1	This
C2764T †	R922W	C-terminus	12	1	This
insG2775-2776 †	G925fs/13	C-terminus	12	1	This
del2906 †	P968fs/4	C-terminus	12	1	This

Nucleotide Change	Coding Effect	Position	Exon	Number of RW Families	Study
del2959-2960†	P986fs/ 130	C-terminus	12	1	This
C3040T †	R1014X	C-terminus	13	2	This
del3094 †	G1031fs/ 24	C-terminus	13	1	This
insG3107-3108	G1036fs/ 82	C-terminus	13	1	Berthet et al., 1999
insC3303-3304 †	P1101fs	C-terminus	14	1	This

* - all characters same as in Table 2

Table 4

Summary of All *SCN5A* Mutations

Nucleotide Change	Coding Effect	Position	Exon	Number of RW Families	Study
G3340A †	D1114N	DII/DIII	18	1	This
C3911T	T1304M	DIII/S4	22	1	Wattanasirichaigoon et al., 1999
A3974G	N1325S	DIII/S4/S5	23	1	Wang et al., 1995b
C4501G †	L1501V	DIII/DIV	26	1	This
del4511-4519	del1505 - 1507	DIII/DIV	26	4	Wang et al., 1995a; Wang et al., 1995b
del4850-4852 †	delF1617	DIV/S3/S4	28	1	This
G4868A	R1623Q	DIV/S4	28	2	This; Makita et al., 1998
G4868T †	R1623L	DIV/S4	28	1	This

Nucleotide Change	Coding Effect	Position	Exon	Number of RW Families	Study
G4931A	R1644H	DIV/S4	28	2	This; Wang et al., 1995b
C4934T	T1645M	DIV/S4	28	1	Wattanasirichaigoon et al., 1999
G5350A †	E1784K	C-terminus	28	2	This; Wei et al., 1999
G5360A †	S1787N	C-terminus	28	1	This
A5369G	D1790G	C-terminus	28	1	An et al., 1998
insTGA 5385-5386	insD1795 -1796	C-terminus	28	1	Bezzina et al., 1999

* - all characters same as in Table 2. Fifty individuals with suspected abnormalities in I_{Na} were screened for all *SCN5A* exons. All individuals were screened for exons 23-28.

Table 5

Summary of All *KCNE1* Mutations*

Nucleotide Change	Coding Effect	Position	Exon	Number of Families	Study
C20T	T7I	N-terminus	3	1 JLN	Schulze-Bahr et al., 1997
G95A †	R32H	N-terminus	3	1	This
G139T	V47F	S1	3	1 JLN	Bianchi et al., 1999
TG151-152AT	L51H	S1	3	1 JLN	Bianchi et al., 1999
A172C/TG 176-177CT	TL58-59PP	S1	3	1 JLN	Tyson et al., 1997
C221T	S74L	C-terminus	3	1	Splawski et al., 1997a

Nucleotide Change	Coding Effect	Position	Exon	Number of Families	Study
G226A	D76N	C-terminus	3	1 JLN, 1 RW, 1 (JLN + RW)	Splawski et al., 1997a; Tyson et al., 1997; Duggal et al., 1998
T259C	W87R	C-terminus	3	1	Bianchi et al., 1999
C292T †	R98W	C-terminus	3	1	This
C379A †	P127T	C-terminus	3	1	This

* - all characters same as in Table 2

Table 6

Summary of All *KCNE2* Mutations

Nucleotide Change	Coding Effect	Position	Exon	Number of Families	Study
C25G	Q9E	N-terminus	1	1	Abbott et al., 1999
T161T	M54T	S1	1	1	Abbott et al., 1999
T170C	I57T	S1	1	1	Abbott et al., 1999

Table 7

Mutations by Type

Type	*KVLQT1*	*HERG*	*SCN5A*	*KCNE1*	*KCNE2*	Total
Missense	59	52	9	5	3	128
Nonsense	6	5	0	0	0	11
AA deletion*	2	2	5	0	0	9
Frameshift	1	16	0	0	0	17
Splice	7	5	0	0	0	12
Total	75	80	14	5	3	177

* - AA denotes amino acid

Table 8

Mutations by Position

Gene **Protein** **Position**	***KVLQT1*** **KVLQT1**	***HERG*** **HERG**	***SCN5A*** **SCN5A**	***KCNE1*** **minK**	***KCNE2*** **MiRP1**	**Total**
Extracellular	0	7	1	1	1	10
Transmembrane	33	13	5	0	2	53
Pore	9	12	0	N/A	N/A	21
Intracellular	33	48	8	4	0	93
Total	75	80	14	5	3	177

While the invention has been disclosed in this patent application by reference to the details of preferred embodiments of the invention, it is to be understood that the disclosure is intended in an illustrative rather than in a limiting sense, as it is contemplated that modifications will readily occur to those skilled in the art, within the spirit of the invention and the scope of the appended claims.

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U.S. Patent No. 5,691,198

U.S. Patent No. 5,735,500

U.S. Patent No. 5,747,469

WHAT IS CLAIMED IS:

1. An isolated DNA comprising a sequence of SEQ ID NO:1 as altered by one or more mutations selected from the group consisting of A332G, G478A, G521A, G535A, G580C, C727T, T742C, T797C, G921+1T, A922-2C, G928A, C1046G, C1066T, G1097A, C1172T, C1343G, C1588T, C1697T, C1747T and G1781A.

2. A nucleic acid probe specifically hybridizable to a human mutated *KVLQT1* and not to wild-type DNA, said mutated *KVLQT1* comprising a mutation of SEQ ID NO:1 selected from the group consisting of A332G, G478A, G521A, G535A, G580C, C727T, T742C, T797C, G921+1T, A922-2C, G928A, C1046G, C1066T, G1097A, C1172T, C1343G, C1588T, C1697T, C1747T and G1781A.

3. A method for detecting a mutation in *KVLQT1* said mutation selected from the group consisting of A332G, G478A, G521A, G535A, G580C, C727T, T742C, T797C, G921+1T, A922-2C, G928A, C1046G, C1066T, G1097A, C1172T, C1343G, C1588T, C1697T, C1747T and G1781A which comprises analyzing a sequence of said gene or RNA from a human sample or analyzing the sequence of cDNA made from mRNA from said sample.

4. The method of claim 3 wherein said mutation is detected by a method selected from the group consisting of:

a) hybridizing a probe specific for one of said mutations to RNA isolated from said human sample and detecting the presence of a hybridization product, wherein the presence of said product indicates the presence of said mutation in the sample;

b) hybridizing a probe specific for one of said mutations to cDNA made from RNA isolated from said sample and detecting the presence of a hybridization product, wherein the presence of said product indicates the presence of said mutation in the sample;

c) hybridizing a probe specific for one of said mutations to genomic DNA isolated from said sample and detecting the presence of a hybridization product, wherein the presence of said product indicates the presence of said mutation in the sample;

d) amplifying all or part of said gene in said sample using a set of primers to produce amplified nucleic acids and sequencing the amplified nucleic acids;

e) amplifying part of said gene in said sample using a primer specific for one of said mutations and detecting the presence of an amplified product, wherein the presence of said product indicates the presence of said mutation in the sample;

f) molecularly cloning all or part of said gene in said sample to produce a cloned nucleic acid and sequencing the cloned nucleic acid;

g) amplifying said gene to produce amplified nucleic acids, hybridizing the amplified nucleic acids to a DNA probe specific for one of said mutations and detecting the presence of a hybridization product, wherein the presence of said product indicates the presence of said mutation;

h) forming single-stranded DNA from a gene fragment of said gene from said human sample and single-stranded DNA from a corresponding fragment of a wild-type gene, electrophoresing said single-stranded DNAs on a non-denaturing polyacrylamide gel and comparing the mobility of said single-stranded DNAs on said gel to determine if said single-stranded DNA from said sample is shifted relative to wild-type and sequencing said single-stranded DNA having a shift in mobility;

i) forming a heteroduplex consisting of a first strand of nucleic acid selected from the group consisting of a genomic DNA fragment isolated from said sample, an RNA fragment isolated from said sample and a cDNA fragment made from mRNA from said sample and a second strand of a nucleic acid consisting of a corresponding human wild-type gene fragment, analyzing for the presence of a mismatch in said heteroduplex, and sequencing said first strand of nucleic acid having a mismatch;

j) forming single-stranded DNA from said gene of said human sample and from a corresponding fragment of an allele specific for one of said mutations, electrophoresing said single-stranded DNAs on a non-denaturing polyacrylamide gel and comparing the mobility of said single-stranded DNAs on said gel to determine if said single-stranded DNA from said sample is shifted relative to said allele, wherein no shift in electrophoretic mobility of the single-stranded DNA relative to the allele indicates the presence of said mutation in said sample; and

k) forming a heteroduplex consisting of a first strand of nucleic acid selected from the group consisting of a genomic DNA fragment of said gene isolated from said sample, an RNA fragment isolated from said sample and a cDNA fragment made from mRNA from said sample and a second strand of a nucleic acid consisting of a corresponding gene allele fragment specific for one of said mutations and analyzing for the presence of a mismatch in said heteroduplex, wherein no mismatch indicates the presence of said mutation.

5. A method according to claim 4 wherein hybridization is performed *in situ*.

6. An isolated human polypeptide encoded by *KVLQT1* comprising a mutation of SEQ ID NO:2 selected from the group consisting of Y111C, E160K, R174H, G179S, A194P, R243C, W248R, L266P, V307sp, V310I, S349W, Q356X, R366Q, T391I, P448R, Q530X, S566F, R583C and R594Q.

7. An antibody capable of binding the polypeptide of claim 6 but incapable of binding a wild-type polypeptide.

8. An antibody according to claim 7 wherein said antibody is a monoclonal antibody.

9. A method of assessing a risk in a human subject for long QT syndrome which comprises screening said subject for a mutation in *KVLQT1* by comparing the sequence of said *KVLQT1* or its expression products isolated from a tissue sample of said subject with a wild-type sequence of said *KVLQT1* or its expression products, wherein a mutation in the sequence of the subject indicates a risk for long QT syndrome.

10. The method of claim 9 wherein said expression product is selected from mRNA of said gene or a polypeptide encoded by said gene.

11. The method of claim 9 wherein one or more of the following procedures is carried out:

(a) observing shifts in electrophoretic mobility of single-stranded DNA from said sample on non-denaturing polyacrylamide gels;

(b) hybridizing a probe to genomic DNA isolated from said sample under conditions suitable for hybridization of said probe to said gene;

(c) determining hybridization of an allele-specific probe to genomic DNA from said sample;

(d) amplifying all or part of said gene from said sample to produce an amplified sequence and sequencing the amplified sequence;

(e) determining by nucleic acid amplification the presence of a specific mutant allele in said sample;

(f) molecularly cloning all or part of said gene from said sample to produce a cloned sequence and sequencing the cloned sequence;

(g) determining whether there is a mismatch between molecules (1) said gene genomic DNA or mRNA isolated from said sample, and (2) a nucleic acid probe complementary to the human wild-type gene DNA, when molecules (1) and (2) are hybridized to each other to form a duplex;

(h) amplification of said gene sequences in said sample and hybridization of the amplified sequences to nucleic acid probes which comprise wild-type gene sequences;

(i) amplification of said gene sequences in said tissue and hybridization of the amplified sequences to nucleic acid probes which comprise said mutant gene sequences;

(j) screening for a deletion mutation;

(k) screening for a point mutation;

(l) screening for an insertion mutation;

(m) determining *in situ* hybridization of said gene in said sample with one or more nucleic acid probes which comprise said gene sequence or a mutant sequence of said gene;

(n) immunoblotting;

(o) immunocytochemistry;

(p) assaying for binding interactions between said gene protein isolated from said tissue and a binding partner capable of specifically binding the polypeptide expression product of a mutant allele and/or a binding partner for the polypeptide; and

(q) assaying for the inhibition of biochemical activity of said binding partner.

12. A nucleic acid probe which hybridizes to the isolated DNA of claim 1 under conditions at which it will not hybridize to wild-type DNA.

13. A method for diagnosing a mutation which causes long QT syndrome comprising hybridizing a probe of claim 12 to a patient's sample of DNA or RNA, the presence of a hybridization signal being indicative of long QT syndrome.

14. A method according to claim 13 wherein the patient's DNA or RNA has been amplified and said amplified DNA or RNA is hybridized with a probe of claim 12.

15. A method according to claim 13 wherein said hybridization is performed *in situ*.

16. A method according to claim 13 wherein said assay is performed using nucleic acid microchip technology.

17. A method for diagnosing a mutation which causes long QT syndrome comprising amplifying a region of gene or RNA for *KVLQT1* and sequencing the amplified gene or RNA wherein long QT syndrome is indicated by any one or more mutations selected from the group consisting of A332G, G478A, G521A, G535A, G580C, C727T, T742C, T797C, G921+1T, A922-2C, G928A, C1046G, C1066T, G1097A, C1172T, C1343G, C1588T, C1697T, C1747T and G1781A.

18. A method for diagnosing a mutation which causes long QT syndrome comprising identifying a mismatch between a patient's DNA or RNA and a wild-type DNA or RNA probe wherein said probe hybridizes to a region of DNA or RNA wherein said region comprises a mutation of SEQ ID NO:1 selected from the group consisting of A332G,

G478A, G521A, G535A, G580C, C727T, T742C, T797C, G921+1T, A922-2C, G928A, C1046G, C1066T, G1097A, C1172T, C1343G, C1588T, C1697T, C1747T and G1781A.

19. The method of claim 18 wherein the mismatch is identified by an RNase assay.

20. A method for diagnosing long QT syndrome said method consisting of an assay for the presence of mutant KVLQT1 polypeptide in a patient by reacting a patient's sample with an antibody of claim 7, the presence of a positive reaction being indicative of long QT syndrome.

21. The method of claim 20 wherein said assay comprises immunoblotting.

22. The method of claim 20 wherein said assay comprises an immunocytochemical technique.

23. A method for diagnosing long QT syndrome, said method comprising analyzing a KVLQT1 polypeptide, a mutation in said polypeptide being indicative of long QT syndrome wherein said mutation is a mutation selected from the group consisting of Y111C, E160K, R174H, G179S, A194P, R243C, W248R, L266P, V307sp, V310I, S349W, Q356X, R366Q, T391I, P448R, Q530X, S566F, R583C and R594Q.

24. A method to screen for drugs which are useful in treating a person with a mutation in *KVLQT1* wherein said mutation is selected from the group consisting of A332G, G478A, G521A, G535A, G580C, C727T, T742C, T797C, G921+1T, A922-2C, G928A, C1046G, C1066T, G1097A, C1172T, C1343G, C1588T, C1697T, C1747T and G1781A, said method comprising:

a) placing a first set of cells expressing KVLQT1 with a mutation, wherein said mutation is selected from the group consisting of Y111C, E160K, R174H, G179S, A194P, R243C, W248R, L266P, V307sp, V310I, S349W, Q356X, R366Q, T391I, P448R, Q530X, S566F, R583C and R594Q, into a bathing solution;

b) inducing a first induced K^+ current in the cells of step (a);

c) measuring said first induced K^+ current;

d) placing a second set of cells expressing wild-type KVLQT1 into a bathing solution

e) inducing a second induced K^+ current in the cells of step (d);

f) measuring said second induced K^+ current;

g) adding a drug to the bathing solution of step (a);

h) inducing a third induced K^+ current in the cells of step (g);

i) measuring said third induced K^+ current; and

j) determining whether the third induced K^+ current is more similar to the second induced K^+ current than is the first induced K^+ current, wherein drugs resulting in a third induced K^+ current which is closer to the second induced K^+ current than is the first induced K^+ current are useful in treating said persons.

25. An isolated DNA encoding a KVLQT1 polypeptide of SEQ ID NO:2 having a mutation selected from the group consisting of Y111C, E160K, R174H, G179S, A194P, R243C, W248R, L266P, V307sp, V310I, S349W, Q356X, R366Q, T391I, P448R, Q530X, S566F, R583C and R594Q.

26. An isolated DNA comprising a sequence of SEQ ID NO:3 as altered by one or more mutations selected from the group consisting of G3340A, C4501G, del4850-4852, G4868T, G5349A and G5360A.

27. A nucleic acid probe specifically hybridizable to a human mutated *SCN5A* and not to wild-type DNA, said mutated *SCN5A* comprising a mutation of SEQ ID NO:3 selected from the group consisting of G3340A, C4501G, del4850-4852, G4868T, G5349A and G5360A.

28. A method for detecting a mutation in *SCN5A* said mutation selected from the group consisting of G3340A, C4501G, del4850-4852, G4868T, G5349A and G5360A which comprises analyzing a sequence of said gene or RNA from a human sample or analyzing the sequence of cDNA made from mRNA from said sample.

29. The method of claim 28 wherein said mutation is detected by a method selected from the group consisting of:

a) hybridizing a probe specific for one of said mutations to RNA isolated from said human sample and detecting the presence of a hybridization product, wherein the presence of said product indicates the presence of said mutation in the sample;

b) hybridizing a probe specific for one of said mutations to cDNA made from RNA isolated from said sample and detecting the presence of a hybridization product, wherein the presence of said product indicates the presence of said mutation in the sample;

c) hybridizing a probe specific for one of said mutations to genomic DNA isolated from said sample and detecting the presence of a hybridization product, wherein the presence of said product indicates the presence of said mutation in the sample;

d) amplifying all or part of said gene in said sample using a set of primers to produce amplified nucleic acids and sequencing the amplified nucleic acids;

e) amplifying part of said gene in said sample using a primer specific for one of said mutations and detecting the presence of an amplified product, wherein the presence of said product indicates the presence of said mutation in the sample;

f) molecularly cloning all or part of said gene in said sample to produce a cloned nucleic acid and sequencing the cloned nucleic acid;

g) amplifying said gene to produce amplified nucleic acids, hybridizing the amplified nucleic acids to a DNA probe specific for one of said mutations and detecting the presence of a hybridization product, wherein the presence of said product indicates the presence of said mutation;

h) forming single-stranded DNA from a gene fragment of said gene from said human sample and single-stranded DNA from a corresponding fragment of a wild-type gene, electrophoresing said single-stranded DNAs on a non-denaturing polyacrylamide gel and comparing the mobility of said single-stranded DNAs on said gel to determine if said single-stranded DNA from said sample is shifted relative to wild-type and sequencing said single-stranded DNA having a shift in mobility;

i) forming a heteroduplex consisting of a first strand of nucleic acid selected from the group consisting of a genomic DNA fragment isolated from said sample, an RNA fragment isolated from said sample and a cDNA fragment made from mRNA from said

sample and a second strand of a nucleic acid consisting of a corresponding human wild-type gene fragment, analyzing for the presence of a mismatch in said heteroduplex, and sequencing said first strand of nucleic acid having a mismatch;

j) forming single-stranded DNA from said gene of said human sample and from a corresponding fragment of an allele specific for one of said mutations, electrophoresing said single-stranded DNAs on a non-denaturing polyacrylamide gel and comparing the mobility of said single-stranded DNAs on said gel to determine if said single-stranded DNA from said sample is shifted relative to said allele, wherein no shift in electrophoretic mobility of the single-stranded DNA relative to the allele indicates the presence of said mutation in said sample; and

k) forming a heteroduplex consisting of a first strand of nucleic acid selected from the group consisting of a genomic DNA fragment of said gene isolated from said sample, an RNA fragment isolated from said sample and a cDNA fragment made from mRNA from said sample and a second strand of a nucleic acid consisting of a corresponding gene allele fragment specific for one of said mutations and analyzing for the presence of a mismatch in said heteroduplex, wherein no mismatch indicates the presence of said mutation.

30. A method according to claim 29 wherein hybridization is performed *in situ*.

31. An isolated human polypeptide encoded by *SCN5A* comprising a mutation of SEQ ID NO:4 selected from the group consisting of D1114N, L1501V, delF1617, R1623L, E1784K and S1787N.

32. An antibody capable of binding the polypeptide of claim 31 but incapable of binding a wild-type polypeptide.

33. An antibody according to claim 32 wherein said antibody is a monoclonal antibody.

34. A method of assessing a risk in a human subject for long QT syndrome which comprises screening said subject for a mutation in *SCN5A* by comparing the sequence of said

SCN5A or its expression products isolated from a tissue sample of said subject with a wild-type sequence of said *SCN5A* or its expression products, wherein a mutation in the sequence of the subject indicates a risk for long QT syndrome.

35. The method of claim 34 wherein said expression product is selected from mRNA of said gene or a polypeptide encoded by said gene.

36. The method of claim 34 wherein one or more of the following procedures is carried out:
(a) observing shifts in electrophoretic mobility of single-stranded DNA from said sample on non-denaturing polyacrylamide gels;
(b) hybridizing a probe to genomic DNA isolated from said sample under conditions suitable for hybridization of said probe to said gene;
(c) determining hybridization of an allele-specific probe to genomic DNA from said sample;
(d) amplifying all or part of said gene from said sample to produce an amplified sequence and sequencing the amplified sequence;
(e) determining by nucleic acid amplification the presence of a specific mutant allele in said sample;
(f) molecularly cloning all or part of said gene from said sample to produce a cloned sequence and sequencing the cloned sequence;
(g) determining whether there is a mismatch between molecules (1) said gene genomic DNA or mRNA isolated from said sample, and (2) a nucleic acid probe complementary to the human wild-type gene DNA, when molecules (1) and (2) are hybridized to each other to form a duplex;
(h) amplification of said gene sequences in said sample and hybridization of the amplified sequences to nucleic acid probes which comprise wild-type gene sequences;
(i) amplification of said gene sequences in said tissue and hybridization of the amplified sequences to nucleic acid probes which comprise said mutant gene sequences;
(j) screening for a deletion mutation;
(k) screening for a point mutation;
(l) screening for an insertion mutation;

(m) determining *in situ* hybridization of said gene in said sample with one or more nucleic acid probes which comprise said gene sequence or a mutant sequence of said gene;

(n) immunoblotting;

(o) immunocytochemistry;

(p) assaying for binding interactions between said gene protein isolated from said tissue and a binding partner capable of specifically binding the polypeptide expression product of a mutant allele and/or a binding partner for the polypeptide; and

(q) assaying for the inhibition of biochemical activity of said binding partner.

37. A nucleic acid probe which hybridizes to the isolated DNA of claim 26 under conditions at which it will not hybridize to wild-type DNA.

38. A method for diagnosing a mutation which causes long QT syndrome comprising hybridizing a probe of claim 37 to a patient's sample of DNA or RNA, the presence of a hybridization signal being indicative of long QT syndrome.

39. A method according to claim 38 wherein the patient's DNA or RNA has been amplified and said amplified DNA or RNA is hybridized with a probe of claim 37.

40. A method according to claim 38 wherein said hybridization is performed *in situ*.

41. A method according to claim 38 wherein said assay is performed using nucleic acid microchip technology.

42. A method for diagnosing a mutation which causes long QT syndrome comprising amplifying a region of gene or RNA for *SCN5A* and sequencing the amplified gene or RNA wherein long QT syndrome is indicated by any one or more mutations selected from the group consisting of G3340A, C4501G, del4850-4852, G4868T, G5349A and G5360A.

43. A method for diagnosing a mutation which causes long QT syndrome comprising identifying a mismatch between a patient's DNA or RNA and a wild-type DNA or RNA probe wherein said probe hybridizes to a region of DNA or RNA wherein said region comprises a mutation of SEQ ID NO:3 selected from the group consisting of G3340A, C4501G, del4850-4852, G4868T, G5349A and G5360A.

44. The method of claim 43 wherein the mismatch is identified by an RNase assay.

45. A method for diagnosing long QT syndrome said method consisting of an assay for the presence of mutant SCN5A polypeptide in a patient by reacting a patient's sample with an antibody of claim 32, the presence of a positive reaction being indicative of long QT syndrome.

46. The method of claim 45 wherein said assay comprises immunoblotting.

47. The method of claim 45 wherein said assay comprises an immunocytochemical technique.

48. A method for diagnosing long QT syndrome, said method comprising analyzing a SCN5A polypeptide, a mutation in said polypeptide being indicative of long QT syndrome wherein said mutation is a mutation selected from the group consisting of D1114N, L1501V, delF1617, R1623L, E1784K and S1787N.

49. A method to screen for drugs which are useful in treating a person with a mutation in *SCN5A* wherein said mutation is selected from the group consisting of G3340A, C4501G, del4850-4852, G4868T, G5349A and G5360A, said method comprising:

a) placing a first set of cells expressing SCN5A with a mutation, wherein said mutation is selected from the group consisting of D1114N, L1501V, delF1617, R1623L, E1784K and S1787N, into a bathing solution;

b) inducing a first induced Na^+ current in the cells of step (a);

c) measuring said first induced Na^+ current;

d) placing a second set of cells expressing wild-type SCN5A into a bathing solution;

e) inducing a second induced Na^+ current in the cells of step (d);

f) measuring said second induced Na^+ current;

g) adding a drug to the bathing solution of step (a);

h) inducing a third induced Na^+ current in the cells in step (g);

i) measuring said third induced Na^+ current; and

j) determining whether the third induced Na^+ current is more similar to the second induced Na^+ current than is the first induced Na^+ current, wherein drugs resulting in a third induced Na^+ current which is closer to the second induced Na^+ current than is the first induced Na^+ current are useful in treating said persons.

50. An isolated DNA encoding an SCN5A polypeptide of SEQ ID NO:4 having a mutation selected from the group consisting of D1114N, L1501V, delF1617, R1623L, E1784K and S1787N.

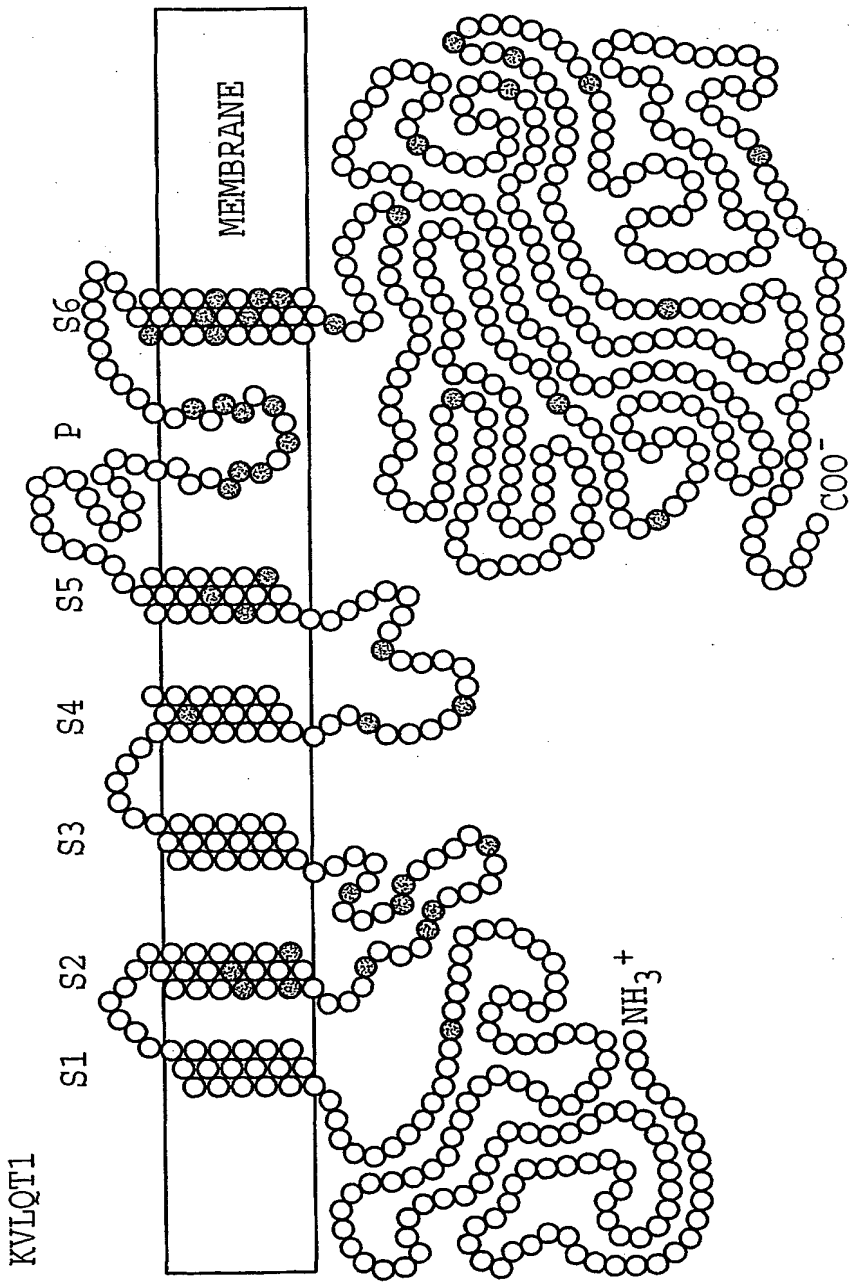

FIG. 1

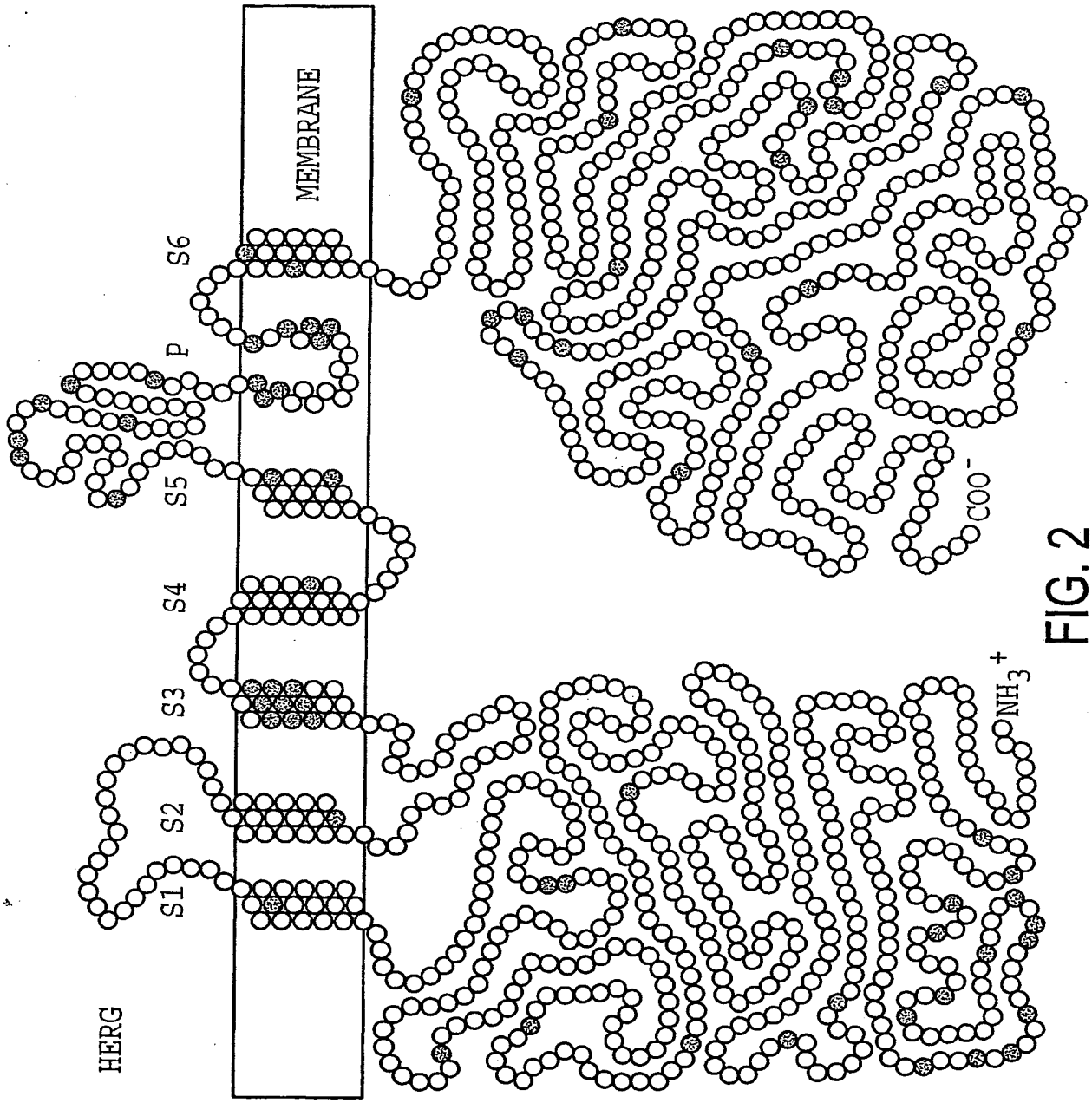

FIG. 2

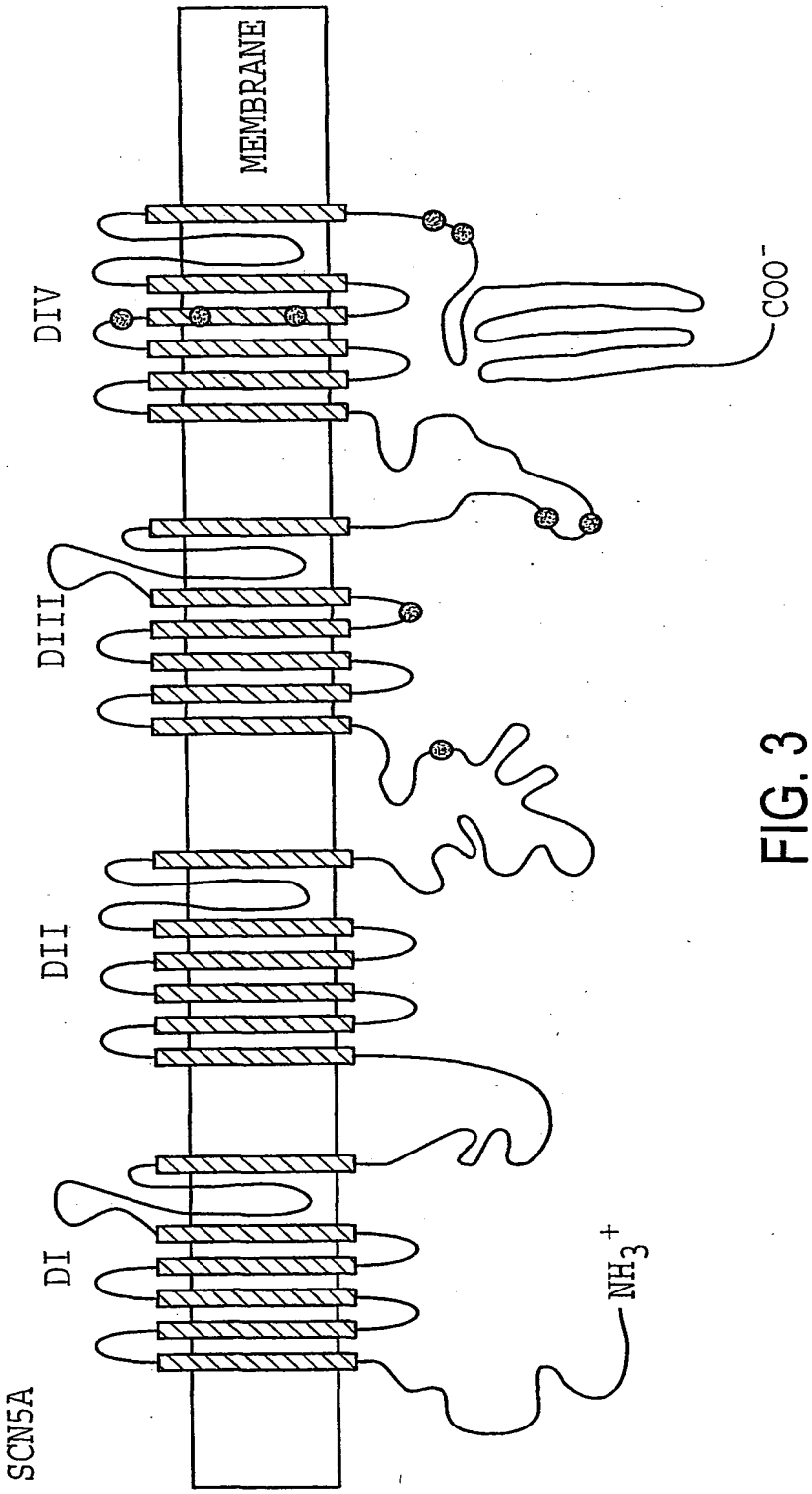

FIG. 3

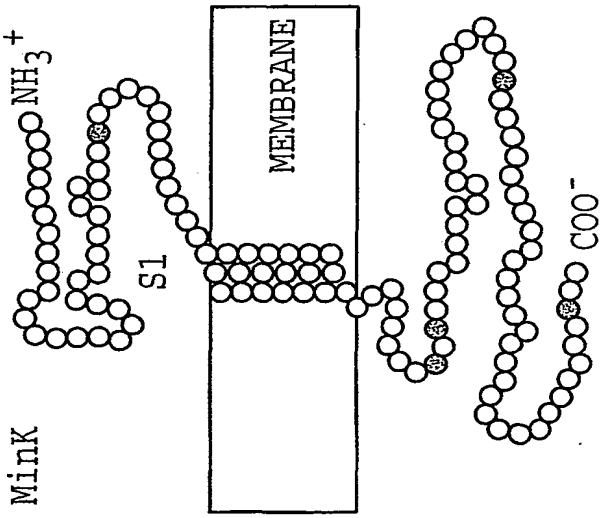

FIG. 4

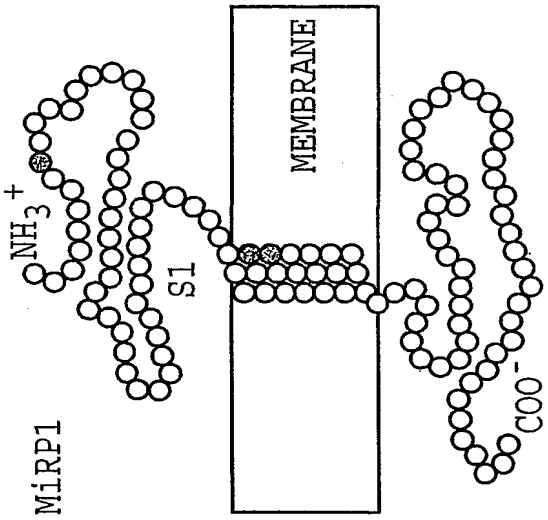

FIG. 5

SEQUENCE LISTING

<110> Splawski, Igor
Keating, Mark T.
University of Utah Research Foundation

<120> ALTERATIONS IN THE LONG QT SYNDROME GENES KVLQT1 AND SCN5A AND METHODS FOR DETECTING SAME

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<140> Not yet assigned
<141> 2000-08-09

<150> 60/190,057
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<151> 1999-08-09

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  1               5                  10                  15

tgg ggc cgc ctg cca ggc gcc cgg cgg ggc agc gcg ggc ctg gcc aag   96
Trp Gly Arg Leu Pro Gly Ala Arg Arg Gly Ser Ala Gly Leu Ala Lys
            20                  25                  30

aag tgc ccc ttc tcg ctg gag ctg gcg gag ggc ggc ccg gcg ggc ggc   144
Lys Cys Pro Phe Ser Leu Glu Leu Ala Glu Gly Gly Pro Ala Gly Gly
        35                  40                  45

gcg ctc tac gcg ccc atc gcg ccc ggc gcc cca ggt ccc gcg ccc cct   192
Ala Leu Tyr Ala Pro Ile Ala Pro Gly Ala Pro Gly Pro Ala Pro Pro
    50                  55                  60

gcg tcc ccg gcc gcg ccc gcc gcg ccc cca gtt gcc tcc gac ctt ggc   240
Ala Ser Pro Ala Ala Pro Ala Ala Pro Pro Val Ala Ser Asp Leu Gly
65                  70                  75                  80

ccg cgg ccg ccg gtg agc cta gac ccg cgc gtc tcc atc tac agc acg   288
Pro Arg Pro Pro Val Ser Leu Asp Pro Arg Val Ser Ile Tyr Ser Thr
                85                  90                  95

cgc cgc ccg gtg ttg gcg cgc acc cac gtc cag ggc cgc gtc tac aac   336
Arg Arg Pro Val Leu Ala Arg Thr His Val Gln Gly Arg Val Tyr Asn
            100                 105                 110
```

```
ttc ctc gag cgt ccc acc ggc tgg aaa tgc ttc gtt tac cac ttc gcc   384
Phe Leu Glu Arg Pro Thr Gly Trp Lys Cys Phe Val Tyr His Phe Ala
        115                 120                 125

gtc ttc ctc atc gtc ctg gtc tgc ctc atc ttc agc gtg ctg tcc acc   432
Val Phe Leu Ile Val Leu Val Cys Leu Ile Phe Ser Val Leu Ser Thr
    130                 135                 140

atc gag cag tat gcc gcc ctg gcc acg ggg act ctc ttc tgg atg gag   480
Ile Glu Gln Tyr Ala Ala Leu Ala Thr Gly Thr Leu Phe Trp Met Glu
145                 150                 155                 160

atc gtg ctg gtg gtg ttc ttc ggg acg gag tac gtg gtc cgc ctc tgg   528
Ile Val Leu Val Val Phe Phe Gly Thr Glu Tyr Val Val Arg Leu Trp
                165                 170                 175

tcc gcc ggc tgc cgc agc aag tac gtg ggc ctc tgg ggg cgg ctg cgc   576
Ser Ala Gly Cys Arg Ser Lys Tyr Val Gly Leu Trp Gly Arg Leu Arg
            180                 185                 190

ttt gcc cgg aag ccc att tcc atc atc gac ctc atc gtg gtc gtg gcc   624
Phe Ala Arg Lys Pro Ile Ser Ile Ile Asp Leu Ile Val Val Val Ala
        195                 200                 205

tcc atg gtg gtc ctc tgc gtg ggc tcc aag ggg cag gtg ttt gcc acg   672
Ser Met Val Val Leu Cys Val Gly Ser Lys Gly Gln Val Phe Ala Thr
    210                 215                 220

tcg gcc atc agg ggc atc cgc ttc ctg cag atc ctg agg atg cta cac   720
Ser Ala Ile Arg Gly Ile Arg Phe Leu Gln Ile Leu Arg Met Leu His
225                 230                 235                 240

gtc gac cgc cag gga ggc acc tgg agg ctc ctg ggc tcc gtg gtc ttc   768
Val Asp Arg Gln Gly Gly Thr Trp Arg Leu Leu Gly Ser Val Val Phe
                245                 250                 255

atc cac cgc cag gag ctg ata acc acc ctg tac atc ggc ttc ctg ggc   816
Ile His Arg Gln Glu Leu Ile Thr Thr Leu Tyr Ile Gly Phe Leu Gly
            260                 265                 270

ctc atc ttc tcc tcg tac ttt gtg tac ctg gct gag aag gac gcg gtg   864
Leu Ile Phe Ser Ser Tyr Phe Val Tyr Leu Ala Glu Lys Asp Ala Val
        275                 280                 285

aac gag tca ggc cgc gtg gag ttc ggc agc tac gca gat gcg ctg tgg   912
Asn Glu Ser Gly Arg Val Glu Phe Gly Ser Tyr Ala Asp Ala Leu Trp
    290                 295                 300

tgg ggg gtg gtc aca gtc acc acc atc ggc tat ggg gac aag gtg ccc   960
Trp Gly Val Val Thr Val Thr Thr Ile Gly Tyr Gly Asp Lys Val Pro
305                 310                 315                 320

cag acg tgg gtc ggg aag acc atc gcc tcc tgc ttc tct gtc ttt gcc  1008
Gln Thr Trp Val Gly Lys Thr Ile Ala Ser Cys Phe Ser Val Phe Ala
                325                 330                 335

atc tcc ttc ttt gcg ctc cca gcg ggg att ctt ggc tcg ggg ttt gcc  1056
Ile Ser Phe Phe Ala Leu Pro Ala Gly Ile Leu Gly Ser Gly Phe Ala
            340                 345                 350

ctg aag gtg cag cag aag cag agg cag aag cac ttc aac cgg cag atc  1104
Leu Lys Val Gln Gln Lys Gln Arg Gln Lys His Phe Asn Arg Gln Ile
        355                 360                 365
```

```
ccg gcg gca gcc tca ctc att cag acc gca tgg agg tgc tat gct gcc   1152
Pro Ala Ala Ala Ser Leu Ile Gln Thr Ala Trp Arg Cys Tyr Ala Ala
    370                 375                 380

gag aac ccc gac tcc tcc acc tgg aag atc tac atc cgg aag gcc ccc   1200
Glu Asn Pro Asp Ser Ser Thr Trp Lys Ile Tyr Ile Arg Lys Ala Pro
385                 390                 395                 400

cgg agc cac act ctg ctg tca ccc agc ccc aaa ccc aag aag tct gtg   1248
Arg Ser His Thr Leu Leu Ser Pro Ser Pro Lys Pro Lys Lys Ser Val
                405                 410                 415

gtg gta aag aaa aaa aag ttc aag ctg gac aaa gac aat ggg gtg act   1296
Val Val Lys Lys Lys Lys Phe Lys Leu Asp Lys Asp Asn Gly Val Thr
            420                 425                 430

cct gga gag aag atg ctc aca gtc ccc cat atc acg tgc gac ccc cca   1344
Pro Gly Glu Lys Met Leu Thr Val Pro His Ile Thr Cys Asp Pro Pro
        435                 440                 445

gaa gag cgg cgg ctg gac cac ttc tct gtc gac ggc tat gac agt tct   1392
Glu Glu Arg Arg Leu Asp His Phe Ser Val Asp Gly Tyr Asp Ser Ser
    450                 455                 460

gta agg aag agc cca aca ctg ctg gaa gtg agc atg ccc cat ttc atg   1440
Val Arg Lys Ser Pro Thr Leu Leu Glu Val Ser Met Pro His Phe Met
465                 470                 475                 480

aga acc aac agc ttc gcc gag gac ctg gac ctg gaa ggg gag act ctg   1488
Arg Thr Asn Ser Phe Ala Glu Asp Leu Asp Leu Glu Gly Glu Thr Leu
                485                 490                 495

ctg aca ccc atc acc cac atc tca cag ctg cgg gaa cac cat cgg gcc   1536
Leu Thr Pro Ile Thr His Ile Ser Gln Leu Arg Glu His His Arg Ala
            500                 505                 510

acc att aag gtc att cga cgc atg cag tac ttt gtg gcc aag aag aaa   1584
Thr Ile Lys Val Ile Arg Arg Met Gln Tyr Phe Val Ala Lys Lys Lys
        515                 520                 525

ttc cag caa gcg cgg aag cct tac gat gtg cgg gac gtc att gag cag   1632
Phe Gln Gln Ala Arg Lys Pro Tyr Asp Val Arg Asp Val Ile Glu Gln
    530                 535                 540

tac tcg cag ggc cac ctc aac ctc atg gtg cgc atc aag gag ctg cag   1680
Tyr Ser Gln Gly His Leu Asn Leu Met Val Arg Ile Lys Glu Leu Gln
545                 550                 555                 560

agg agg ctg gac cag tcc att ggg aag ccc tca ctg ttc atc tcc gtc   1728
Arg Arg Leu Asp Gln Ser Ile Gly Lys Pro Ser Leu Phe Ile Ser Val
                565                 570                 575

tca gaa aag agc aag gat cgc ggc agc aac acg atc ggc gcc cgc ctg   1776
Ser Glu Lys Ser Lys Asp Arg Gly Ser Asn Thr Ile Gly Ala Arg Leu
            580                 585                 590

aac cga gta gaa gac aag gtg acg cag ctg gac cag agg ctg gca ctc   1824
Asn Arg Val Glu Asp Lys Val Thr Gln Leu Asp Gln Arg Leu Ala Leu
        595                 600                 605

atc acc gac atg ctt cac cag ctg ctc tcc ttg cac ggt ggc agc acc   1872
Ile Thr Asp Met Leu His Gln Leu Leu Ser Leu His Gly Gly Ser Thr
    610                 615                 620
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ccc ggc agc ggc ggc ccc ccc aga gag ggc ggg gcc cac atc acc cag   1920
Pro Gly Ser Gly Gly Pro Pro Arg Glu Gly Gly Ala His Ile Thr Gln
625                 630                 635                 640

ccc tgc ggc agt ggc ggc tcc gtc gac cct gag ctc ttc ctg ccc agc   1968
Pro Cys Gly Ser Gly Gly Ser Val Asp Pro Glu Leu Phe Leu Pro Ser
                645                 650                 655

aac acc ctg ccc acc tac gag cag ctg acc gtg ccc agg agg ggc ccc   2016
Asn Thr Leu Pro Thr Tyr Glu Gln Leu Thr Val Pro Arg Arg Gly Pro
            660                 665                 670

gat gag ggg tcc                                                  2028
Asp Glu Gly Ser
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            20                  25                  30

Lys Cys Pro Phe Ser Leu Glu Leu Ala Glu Gly Gly Pro Ala Gly Gly
        35                  40                  45

Ala Leu Tyr Ala Pro Ile Ala Pro Gly Ala Pro Gly Pro Ala Pro Pro
    50                  55                  60

Ala Ser Pro Ala Ala Pro Ala Ala Pro Pro Val Ala Ser Asp Leu Gly
65                  70                  75                  80

Pro Arg Pro Pro Val Ser Leu Asp Pro Arg Val Ser Ile Tyr Ser Thr
                85                  90                  95

Arg Arg Pro Val Leu Ala Arg Thr His Val Gln Gly Arg Val Tyr Asn
            100                 105                 110

Phe Leu Glu Arg Pro Thr Gly Trp Lys Cys Phe Val Tyr His Phe Ala
        115                 120                 125

Val Phe Leu Ile Val Leu Val Cys Leu Ile Phe Ser Val Leu Ser Thr
    130                 135                 140

Ile Glu Gln Tyr Ala Ala Leu Ala Thr Gly Thr Leu Phe Trp Met Glu
145                 150                 155                 160

Ile Val Leu Val Val Phe Phe Gly Thr Glu Tyr Val Val Arg Leu Trp
                165                 170                 175

Ser Ala Gly Cys Arg Ser Lys Tyr Val Gly Leu Trp Gly Arg Leu Arg
            180                 185                 190

Phe Ala Arg Lys Pro Ile Ser Ile Ile Asp Leu Ile Val Val Val Ala
        195                 200                 205
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Ser Met Val Val Leu Cys Val Gly Ser Lys Gly Gln Val Phe Ala Thr
210 215 220

Ser Ala Ile Arg Gly Ile Arg Phe Leu Gln Ile Leu Arg Met Leu His
225 230 235 240

Val Asp Arg Gln Gly Gly Thr Trp Arg Leu Leu Gly Ser Val Val Phe
245 250 255

Ile His Arg Gln Glu Leu Ile Thr Thr Leu Tyr Ile Gly Phe Leu Gly
260 265 270

Leu Ile Phe Ser Ser Tyr Phe Val Tyr Leu Ala Glu Lys Asp Ala Val
275 280 285

Asn Glu Ser Gly Arg Val Glu Phe Gly Ser Tyr Ala Asp Ala Leu Trp
290 295 300

Trp Gly Val Val Thr Val Thr Thr Ile Gly Tyr Gly Asp Lys Val Pro
305 310 315 320

Gln Thr Trp Val Gly Lys Thr Ile Ala Ser Cys Phe Ser Val Phe Ala
325 330 335

Ile Ser Phe Phe Ala Leu Pro Ala Gly Ile Leu Gly Ser Gly Phe Ala
340 345 350

Leu Lys Val Gln Gln Lys Gln Arg Gln Lys His Phe Asn Arg Gln Ile
355 360 365

Pro Ala Ala Ala Ser Leu Ile Gln Thr Ala Trp Arg Cys Tyr Ala Ala
370 375 380

Glu Asn Pro Asp Ser Ser Thr Trp Lys Ile Tyr Ile Arg Lys Ala Pro
385 390 395 400

Arg Ser His Thr Leu Leu Ser Pro Ser Pro Lys Pro Lys Lys Ser Val
405 410 415

Val Val Lys Lys Lys Lys Phe Lys Leu Asp Lys Asp Asn Gly Val Thr
420 425 430

Pro Gly Glu Lys Met Leu Thr Val Pro His Ile Thr Cys Asp Pro Pro
435 440 445

Glu Glu Arg Arg Leu Asp His Phe Ser Val Asp Gly Tyr Asp Ser Ser
450 455 460

Val Arg Lys Ser Pro Thr Leu Leu Glu Val Ser Met Pro His Phe Met
465 470 475 480

Arg Thr Asn Ser Phe Ala Glu Asp Leu Asp Leu Glu Gly Glu Thr Leu
485 490 495

Leu Thr Pro Ile Thr His Ile Ser Gln Leu Arg Glu His His Arg Ala
500 505 510

Thr Ile Lys Val Ile Arg Arg Met Gln Tyr Phe Val Ala Lys Lys Lys
515 520 525

Phe Gln Gln Ala Arg Lys Pro Tyr Asp Val Arg Asp Val Ile Glu Gln
530 535 540

```
Tyr Ser Gln Gly His Leu Asn Leu Met Val Arg Ile Lys Glu Leu Gln
545                 550                 555                 560

Arg Arg Leu Asp Gln Ser Ile Gly Lys Pro Ser Leu Phe Ile Ser Val
                565                 570                 575

Ser Glu Lys Ser Lys Asp Arg Gly Ser Asn Thr Ile Gly Ala Arg Leu
            580                 585                 590

Asn Arg Val Glu Asp Lys Val Thr Gln Leu Asp Gln Arg Leu Ala Leu
        595                 600                 605

Ile Thr Asp Met Leu His Gln Leu Leu Ser Leu His Gly Gly Ser Thr
    610                 615                 620

Pro Gly Ser Gly Gly Pro Pro Arg Glu Gly Gly Ala His Ile Thr Gln
625                 630                 635                 640

Pro Cys Gly Ser Gly Gly Ser Val Asp Pro Glu Leu Phe Leu Pro Ser
                645                 650                 655

Asn Thr Leu Pro Thr Tyr Glu Gln Leu Thr Val Pro Arg Arg Gly Pro
            660                 665                 670

Asp Glu Gly Ser
        675
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Met Ala Asn Phe Leu Leu Pro Arg Gly Thr Ser Ser Phe Arg Arg Phe
  1               5                  10                  15

aca cgg gag tcc ctg gca gcc atc gag aag cgc atg gcg gag aag caa   96
Thr Arg Glu Ser Leu Ala Ala Ile Glu Lys Arg Met Ala Glu Lys Gln
             20                  25                  30

gcc cgc ggc tca acc acc ttg cag gag agc cga gag ggg ctg ccc gag   144
Ala Arg Gly Ser Thr Thr Leu Gln Glu Ser Arg Glu Gly Leu Pro Glu
         35                  40                  45

gag gag gct ccc cgg ccc cag ctg gac ctg cag gcc tcc aaa aag ctg   192
Glu Glu Ala Pro Arg Pro Gln Leu Asp Leu Gln Ala Ser Lys Lys Leu
     50                  55                  60

cca gat ctc tat ggc aat cca ccc caa gag ctc atc gga gag ccc ctg   240
Pro Asp Leu Tyr Gly Asn Pro Pro Gln Glu Leu Ile Gly Glu Pro Leu
 65                  70                  75                  80

gag gac ctg gac ccc ttc tat agc acc caa aag act ttc atc gta ctg   288
Glu Asp Leu Asp Pro Phe Tyr Ser Thr Gln Lys Thr Phe Ile Val Leu
                 85                  90                  95
```

```
aat aaa ggc aag acc atc ttc cgg ttc agt gcc acc aac gcc ttg tat   336
Asn Lys Gly Lys Thr Ile Phe Arg Phe Ser Ala Thr Asn Ala Leu Tyr
            100                 105                 110

gtc ctc agt ccc ttc cac cca gtt cgg aga gcg gct gtg aag att ctg   384
Val Leu Ser Pro Phe His Pro Val Arg Arg Ala Ala Val Lys Ile Leu
        115                 120                 125

gtt cac tcg ctc ttc aac atg ctc atc atg tgc acc atc ctc acc aac   432
Val His Ser Leu Phe Asn Met Leu Ile Met Cys Thr Ile Leu Thr Asn
    130                 135                 140

tgc gtg ttc atg gcc cag cac gac cct cca ccc tgg acc aag tat gtc   480
Cys Val Phe Met Ala Gln His Asp Pro Pro Pro Trp Thr Lys Tyr Val
145                 150                 155                 160

gag tac acc ttc acc gcc att tac acc ttt gag tct ctg gtc aag att   528
Glu Tyr Thr Phe Thr Ala Ile Tyr Thr Phe Glu Ser Leu Val Lys Ile
                165                 170                 175

ctg gct cga gct ttc tgc ctg cac gcg ttc act ttc ctt cgg gac cca   576
Leu Ala Arg Ala Phe Cys Leu His Ala Phe Thr Phe Leu Arg Asp Pro
            180                 185                 190

tgg aac tgg ctg gac ttt agt gtg att atc atg gca tac aca act gaa   624
Trp Asn Trp Leu Asp Phe Ser Val Ile Ile Met Ala Tyr Thr Thr Glu
        195                 200                 205

ttt gtg gac ctg ggc aat gtc tca gcc tta cgc acc ttc cga gtc ctc   672
Phe Val Asp Leu Gly Asn Val Ser Ala Leu Arg Thr Phe Arg Val Leu
    210                 215                 220

cgg gcc ctg aaa act ata tca gtc att tca ggg ctg aag acc atc gtg   720
Arg Ala Leu Lys Thr Ile Ser Val Ile Ser Gly Leu Lys Thr Ile Val
225                 230                 235                 240

ggg gcc ctg atc cag tct gtg aag aag ctg gct gat gtg atg gtc ctc   768
Gly Ala Leu Ile Gln Ser Val Lys Lys Leu Ala Asp Val Met Val Leu
                245                 250                 255

aca gtc ttc tgc ctc agc gtc ttt gcc ctc atc ggc ctg cag ctc ttc   816
Thr Val Phe Cys Leu Ser Val Phe Ala Leu Ile Gly Leu Gln Leu Phe
            260                 265                 270

atg ggc aac cta agg cac aag tgt gtg cgc aac ttc aca gcg ctc aac   864
Met Gly Asn Leu Arg His Lys Cys Val Arg Asn Phe Thr Ala Leu Asn
        275                 280                 285

ggc acc aac ggc tcc gtg gag gcc gac ggc ttg gtc tgg gaa tcc ctg   912
Gly Thr Asn Gly Ser Val Glu Ala Asp Gly Leu Val Trp Glu Ser Leu
    290                 295                 300

gac ctt tac ctc agt gat cca gaa aat tac ctg ctc aag aac ggc acc   960
Asp Leu Tyr Leu Ser Asp Pro Glu Asn Tyr Leu Leu Lys Asn Gly Thr
305                 310                 315                 320

tct gat gtg tta ctg tgt ggg aac agc tct gac gct ggg aca tgt ccg  1008
Ser Asp Val Leu Leu Cys Gly Asn Ser Ser Asp Ala Gly Thr Cys Pro
                325                 330                 335

gag ggc tac cgg tgc cta aag gca ggc gag aac ccc gac cac ggc tac  1056
Glu Gly Tyr Arg Cys Leu Lys Ala Gly Glu Asn Pro Asp His Gly Tyr
            340                 345                 350
```

```
acc agc ttc gat tcc ttt gcc tgg gcc ttt ctt gca ctc ttc cgc ctg   1104
Thr Ser Phe Asp Ser Phe Ala Trp Ala Phe Leu Ala Leu Phe Arg Leu
        355                 360                 365

atg acg cag gac tgc tgg gag cgc ctc tat cag cag acc ctc agg tcc   1152
Met Thr Gln Asp Cys Trp Glu Arg Leu Tyr Gln Gln Thr Leu Arg Ser
    370                 375                 380

gca ggg aag atc tac atg atc ttc ttc atg ctt gtc atc ttc ctg ggg   1200
Ala Gly Lys Ile Tyr Met Ile Phe Phe Met Leu Val Ile Phe Leu Gly
385                 390                 395                 400

tcc ttc tac ctg gtg aac ctg atc ctg gcc gtg gtc gca atg gcc tat   1248
Ser Phe Tyr Leu Val Asn Leu Ile Leu Ala Val Val Ala Met Ala Tyr
                405                 410                 415

gag gag caa aac caa gcc acc atc gct gag acc gag gag aag gaa aag   1296
Glu Glu Gln Asn Gln Ala Thr Ile Ala Glu Thr Glu Glu Lys Glu Lys
            420                 425                 430

cgc ttc cag gag gcc atg gaa atg ctc aag aaa gaa cac gag gcc ctc   1344
Arg Phe Gln Glu Ala Met Glu Met Leu Lys Lys Glu His Glu Ala Leu
        435                 440                 445

acc atc agg ggt gtg gat acc gtg tcc cgt agc tcc ttg gag atg tcc   1392
Thr Ile Arg Gly Val Asp Thr Val Ser Arg Ser Ser Leu Glu Met Ser
    450                 455                 460

cct ttg gcc cca gta aac agc cat gag aga aga agc aag agg aga aaa   1440
Pro Leu Ala Pro Val Asn Ser His Glu Arg Arg Ser Lys Arg Arg Lys
465                 470                 475                 480

cgg atg tct tca gga act gag gag tgt ggg gag gac agg ctc ccc aag   1488
Arg Met Ser Ser Gly Thr Glu Glu Cys Gly Glu Asp Arg Leu Pro Lys
                485                 490                 495

tct gac tca gaa gat ggt ccc aga gca atg aat cat ctc agc ctc acc   1536
Ser Asp Ser Glu Asp Gly Pro Arg Ala Met Asn His Leu Ser Leu Thr
            500                 505                 510

cgt ggc ctc agc agg act tct atg aag cca cgt tcc agc cgc ggg agc   1584
Arg Gly Leu Ser Arg Thr Ser Met Lys Pro Arg Ser Ser Arg Gly Ser
        515                 520                 525

att ttc acc ttt cgc agg cga gac ctg ggt tct gaa gca gat ttt gca   1632
Ile Phe Thr Phe Arg Arg Arg Asp Leu Gly Ser Glu Ala Asp Phe Ala
    530                 535                 540

gat gat gaa aac agc aca gcg cgg gag agc gag agc cac cac aca tca   1680
Asp Asp Glu Asn Ser Thr Ala Arg Glu Ser Glu Ser His His Thr Ser
545                 550                 555                 560

ctg ctg gtg ccc tgg ccc ctg cgc cgg acc agt gcc cag gga cag ccc   1728
Leu Leu Val Pro Trp Pro Leu Arg Arg Thr Ser Ala Gln Gly Gln Pro
                565                 570                 575

agt ccc gga acc tcg gct cct ggc cac gcc ctc cat ggc aaa aag aac   1776
Ser Pro Gly Thr Ser Ala Pro Gly His Ala Leu His Gly Lys Lys Asn
            580                 585                 590

agc act gtg gac tgc aat ggg gtg gtc tca tta ctg ggg gca ggc gac   1824
Ser Thr Val Asp Cys Asn Gly Val Val Ser Leu Leu Gly Ala Gly Asp
        595                 600                 605
```

```
cca gag gcc aca tcc cca gga agc cac ctc ctc cgc cct gtg atg cta   1872
Pro Glu Ala Thr Ser Pro Gly Ser His Leu Leu Arg Pro Val Met Leu
    610                 615                 620

gag cac ccg cca gac acg acc acg cca tcg gag gag cca ggc ggc ccc   1920
Glu His Pro Pro Asp Thr Thr Thr Pro Ser Glu Glu Pro Gly Gly Pro
625                 630                 635                 640

cag atg ctg acc tcc cag gct ccg tgt gta gat ggc ttc gag gag cca   1968
Gln Met Leu Thr Ser Gln Ala Pro Cys Val Asp Gly Phe Glu Glu Pro
                645                 650                 655

gga gca cgg cag cgg gcc ctc agc gca gtc agc gtc ctc aca agc gca   2016
Gly Ala Arg Gln Arg Ala Leu Ser Ala Val Ser Val Leu Thr Ser Ala
            660                 665                 670

ctg gaa gag tta gag gag tct cgc cac aag tgt cca cca tgc tgg aac   2064
Leu Glu Glu Leu Glu Glu Ser Arg His Lys Cys Pro Pro Cys Trp Asn
        675                 680                 685

cgt ctc gcc cag cgc tac ctg atc tgg gag tgc tgc ccg ctg tgg atg   2112
Arg Leu Ala Gln Arg Tyr Leu Ile Trp Glu Cys Cys Pro Leu Trp Met
    690                 695                 700

tcc atc aag cag gga gtg aag ttg gtg gtc atg gac ccg ttt act gac   2160
Ser Ile Lys Gln Gly Val Lys Leu Val Val Met Asp Pro Phe Thr Asp
705                 710                 715                 720

ctc acc atc act atg tgc atc gta ctc aac aca ctc ttc atg gcg ctg   2208
Leu Thr Ile Thr Met Cys Ile Val Leu Asn Thr Leu Phe Met Ala Leu
                725                 730                 735

gag cac tac aac atg aca agt gaa ttc gag gag atg ctg cag gtc gga   2256
Glu His Tyr Asn Met Thr Ser Glu Phe Glu Glu Met Leu Gln Val Gly
            740                 745                 750

aac ctg gtc ttc aca ggg att ttc aca gca gag atg acc ttc aag atc   2304
Asn Leu Val Phe Thr Gly Ile Phe Thr Ala Glu Met Thr Phe Lys Ile
        755                 760                 765

att gcc ctc gac ccc tac tac tac ttc caa cag ggc tgg aac atc ttc   2352
Ile Ala Leu Asp Pro Tyr Tyr Tyr Phe Gln Gln Gly Trp Asn Ile Phe
    770                 775                 780

gac agc atc atc gtc atc ctt agc ctc atg gag ctg ggc ctg tcc cgc   2400
Asp Ser Ile Ile Val Ile Leu Ser Leu Met Glu Leu Gly Leu Ser Arg
785                 790                 795                 800

atg agc aac ttg tcg gtg ctg cgc tcc ttc cgc ctg ctg cgg gtc ttc   2448
Met Ser Asn Leu Ser Val Leu Arg Ser Phe Arg Leu Leu Arg Val Phe
                805                 810                 815

aag ctg gcc aaa tca tgg ccc acc ctg aac aca ctc atc aag atc atc   2496
Lys Leu Ala Lys Ser Trp Pro Thr Leu Asn Thr Leu Ile Lys Ile Ile
            820                 825                 830

ggg aac tca gtg ggg gca ctg ggg aac ctg aca ctg gtg cta gcc atc   2544
Gly Asn Ser Val Gly Ala Leu Gly Asn Leu Thr Leu Val Leu Ala Ile
        835                 840                 845

atc gtg ttc atc ttt gct gtg gtg ggc atg cag ctc ttt ggc aag aac   2592
Ile Val Phe Ile Phe Ala Val Val Gly Met Gln Leu Phe Gly Lys Asn
    850                 855                 860
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tac tcg gag ctg agg gac agc gac tca ggc ctg ctg cct cgc tgg cac   2640
Tyr Ser Glu Leu Arg Asp Ser Asp Ser Gly Leu Leu Pro Arg Trp His
865                 870                 875                 880

atg atg gac ttc ttt cat gcc ttc cta atc atc ttc cgc atc ctc tgt   2688
Met Met Asp Phe Phe His Ala Phe Leu Ile Ile Phe Arg Ile Leu Cys
                885                 890                 895

gga gag tgg atc gag acc atg tgg gac tgc atg gag gtg tcg ggg cag   2736
Gly Glu Trp Ile Glu Thr Met Trp Asp Cys Met Glu Val Ser Gly Gln
            900                 905                 910

tca tta tgc ctg ctg gtc ttc ttg ctt gtt atg gtc att ggc aac ctt   2784
Ser Leu Cys Leu Leu Val Phe Leu Leu Val Met Val Ile Gly Asn Leu
        915                 920                 925

gtg gtc ctg aat ctc ttc ctg gcc ttg ctg ctc agc tcc ttc agt gca   2832
Val Val Leu Asn Leu Phe Leu Ala Leu Leu Leu Ser Ser Phe Ser Ala
    930                 935                 940

gac aac ctc aca gcc cct gat gag gac aga gag atg aac aac ctc cag   2880
Asp Asn Leu Thr Ala Pro Asp Glu Asp Arg Glu Met Asn Asn Leu Gln
945                 950                 955                 960

ctg gcc ctg gcc cgc atc cag agg ggc ctg cgc ttt gtc aag cgg acc   2928
Leu Ala Leu Ala Arg Ile Gln Arg Gly Leu Arg Phe Val Lys Arg Thr
                965                 970                 975

acc tgg gat ttc tgc tgt ggt ctc ctg cgg cac cgg cct cag aag ccc   2976
Thr Trp Asp Phe Cys Cys Gly Leu Leu Arg His Arg Pro Gln Lys Pro
            980                 985                 990

gca gcc ctt gcc gcc cag ggc cag ctg ccc agc tgc att gcc acc ccc   3024
Ala Ala Leu Ala Ala Gln Gly Gln Leu Pro Ser Cys Ile Ala Thr Pro
        995                1000                1005

tac tcc ccg cca ccc cca gag acg gag aag gtg cct ccc acc cgc aag   3072
Tyr Ser Pro Pro Pro Pro Glu Thr Glu Lys Val Pro Pro Thr Arg Lys
   1010                1015                1020

gaa aca cag ttt gag gaa ggc gag caa cca ggc cag ggc acc ccc ggg   3120
Glu Thr Gln Phe Glu Glu Gly Glu Gln Pro Gly Gln Gly Thr Pro Gly
1025                1030                1035                1040

gat cca gag ccc gtg tgt gtg ccc atc gct gtg gcc gag tca gac aca   3168
Asp Pro Glu Pro Val Cys Val Pro Ile Ala Val Ala Glu Ser Asp Thr
                1045                1050                1055

gat gac caa gaa gag gat gag gag aac agc ctg ggc acg gag gag gag   3216
Asp Asp Gln Glu Glu Asp Glu Glu Asn Ser Leu Gly Thr Glu Glu Glu
            1060                1065                1070

tcc agc aag cag cag gaa tcc cag cct gtg tcc ggc tgg ccc aga ggc   3264
Ser Ser Lys Gln Gln Glu Ser Gln Pro Val Ser Gly Trp Pro Arg Gly
        1075                1080                1085

cct ccg gat tcc agg acc tgg agc cag gtg tca gcg act gcc tcc tct   3312
Pro Pro Asp Ser Arg Thr Trp Ser Gln Val Ser Ala Thr Ala Ser Ser
   1090                1095                1100

gag gcc gag gcc agt gca tct cag gcc gac tgg cgg cag cag tgg aaa   3360
Glu Ala Glu Ala Ser Ala Ser Gln Ala Asp Trp Arg Gln Gln Trp Lys
1105                1110                1115                1120
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gcg gaa ccc cag gcc cca ggg tgc ggt gag acc cca gag gac agt tgc   3408
Ala Glu Pro Gln Ala Pro Gly Cys Gly Glu Thr Pro Glu Asp Ser Cys
                1125                1130                1135

tcc gag ggc agc aca gca gac atg acc aac acc gct gag ctc ctg gag   3456
Ser Glu Gly Ser Thr Ala Asp Met Thr Asn Thr Ala Glu Leu Leu Glu
            1140                1145                1150

cag atc cct gac ctc ggc cag gat gtc aag gac cca gag gac tgc ttc   3504
Gln Ile Pro Asp Leu Gly Gln Asp Val Lys Asp Pro Glu Asp Cys Phe
        1155                1160                1165

act gaa ggc tgt gtc cgg cgc tgt ccc tgc tgt gcg gtg gac acc aca   3552
Thr Glu Gly Cys Val Arg Arg Cys Pro Cys Cys Ala Val Asp Thr Thr
    1170                1175                1180

cag gcc cca ggg aag gtc tgg tgg cgg ttg cgc aag acc tgc tac cac   3600
Gln Ala Pro Gly Lys Val Trp Trp Arg Leu Arg Lys Thr Cys Tyr His
1185                1190                1195                1200

atc gtg gag cac agc tgg ttc gag aca ttc atc atc ttc atg atc cta   3648
Ile Val Glu His Ser Trp Phe Glu Thr Phe Ile Ile Phe Met Ile Leu
                1205                1210                1215

ctc agc agt gga gcg ctg gcc ttc gag gac atc tac cta gag gag cgg   3696
Leu Ser Ser Gly Ala Leu Ala Phe Glu Asp Ile Tyr Leu Glu Glu Arg
            1220                1225                1230

aag acc atc aag gtt ctg ctt gag tat gcc gac aag atg ttc aca tat   3744
Lys Thr Ile Lys Val Leu Leu Glu Tyr Ala Asp Lys Met Phe Thr Tyr
        1235                1240                1245

gtc ttc gtg ctg gag atg ctg ctc aag tgg gtg gcc tac ggc ttc aag   3792
Val Phe Val Leu Glu Met Leu Leu Lys Trp Val Ala Tyr Gly Phe Lys
    1250                1255                1260

aag tac ttc acc aat gcc tgg tgc tgg ctc gac ttc ctc atc gta gac   3840
Lys Tyr Phe Thr Asn Ala Trp Cys Trp Leu Asp Phe Leu Ile Val Asp
1265                1270                1275                1280

gtc tct ctg gtc agc ctg gtg gcc aac acc ctg ggc ttt gcc gag atg   3888
Val Ser Leu Val Ser Leu Val Ala Asn Thr Leu Gly Phe Ala Glu Met
                1285                1290                1295

ggc ccc atc aag tca ctg cgg acg ctg cgt gca ctc cgt cct ctg aga   3936
Gly Pro Ile Lys Ser Leu Arg Thr Leu Arg Ala Leu Arg Pro Leu Arg
            1300                1305                1310

gct ctg tca cga ttt gag ggc atg agg gtg gtg gtc aat gcc ctg gtg   3984
Ala Leu Ser Arg Phe Glu Gly Met Arg Val Val Val Asn Ala Leu Val
        1315                1320                1325

ggc gcc atc ccg tcc atc atg aac gtc ctc ctc gtc tgc ctc atc ttc   4032
Gly Ala Ile Pro Ser Ile Met Asn Val Leu Leu Val Cys Leu Ile Phe
    1330                1335                1340

tgg ctc atc ttc agc atc atg ggc gtg aac ctc ttt gcg ggg aag ttt   4080
Trp Leu Ile Phe Ser Ile Met Gly Val Asn Leu Phe Ala Gly Lys Phe
1345                1350                1355                1360

ggg agg tgc atc aac cag aca gag gga gac ttg cct ttg aac tac acc   4128
Gly Arg Cys Ile Asn Gln Thr Glu Gly Asp Leu Pro Leu Asn Tyr Thr
                1365                1370                1375
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atc gtg aac aac aag agc cag tgt gag tcc ttg aac ttg acc gga gaa   4176
Ile Val Asn Asn Lys Ser Gln Cys Glu Ser Leu Asn Leu Thr Gly Glu
          1380                1385                1390

ttg tac tgg acc aag gtg aaa gtc aac ttt gac aac gtg ggg gcc ggg   4224
Leu Tyr Trp Thr Lys Val Lys Val Asn Phe Asp Asn Val Gly Ala Gly
      1395                1400                1405

tac ctg gcc ctt ctg cag gtg gca aca ttt aaa ggc tgg atg gac att   4272
Tyr Leu Ala Leu Leu Gln Val Ala Thr Phe Lys Gly Trp Met Asp Ile
  1410                1415                1420

atg tat gca gct gtg gac tcc agg ggg tat gaa gag cag cct cag tgg   4320
Met Tyr Ala Ala Val Asp Ser Arg Gly Tyr Glu Glu Gln Pro Gln Trp
1425                1430                1435                1440

gaa tac aac ctc tac atg tac atc tat ttt gtc att ttc atc atc ttt   4368
Glu Tyr Asn Leu Tyr Met Tyr Ile Tyr Phe Val Ile Phe Ile Ile Phe
              1445                1450                1455

ggg tct ttc ttc acc ctg aac ctc ttt att ggt gtc atc att gac aac   4416
Gly Ser Phe Phe Thr Leu Asn Leu Phe Ile Gly Val Ile Ile Asp Asn
          1460                1465                1470

ttc aac caa cag aag aaa aag tta ggg ggc cag gac atc ttc atg aca   4464
Phe Asn Gln Gln Lys Lys Lys Leu Gly Gly Gln Asp Ile Phe Met Thr
      1475                1480                1485

gag gag cag aag aag tac tac aat gcc atg aag aag ctg ggc tcc aag   4512
Glu Glu Gln Lys Lys Tyr Tyr Asn Ala Met Lys Lys Leu Gly Ser Lys
  1490                1495                1500

aag ccc cag aag ccc atc cca cgg ccc ctg aac aag tac cag ggc ttc   4560
Lys Pro Gln Lys Pro Ile Pro Arg Pro Leu Asn Lys Tyr Gln Gly Phe
1505                1510                1515                1520

ata ttc gac att gtg acc aag cag gcc ttt gac gtc acc atc atg ttt   4608
Ile Phe Asp Ile Val Thr Lys Gln Ala Phe Asp Val Thr Ile Met Phe
              1525                1530                1535

ctg atc tgc ttg aat atg gtg acc atg atg gtg gag aca gat gac caa   4656
Leu Ile Cys Leu Asn Met Val Thr Met Met Val Glu Thr Asp Asp Gln
          1540                1545                1550

agt cct gag aaa atc aac atc ttg gcc aag atc aac ctg ctc ttt gtg   4704
Ser Pro Glu Lys Ile Asn Ile Leu Ala Lys Ile Asn Leu Leu Phe Val
      1555                1560                1565

gcc atc ttc aca ggc gag tgt att gtc aag ctg gct gcc ctg cgc cac   4752
Ala Ile Phe Thr Gly Glu Cys Ile Val Lys Leu Ala Ala Leu Arg His
  1570                1575                1580

tac tac ttc acc aac agc tgg aat atc ttc gac ttc gtg gtt gtc atc   4800
Tyr Tyr Phe Thr Asn Ser Trp Asn Ile Phe Asp Phe Val Val Val Ile
1585                1590                1595                1600

ctc tcc atc gtg ggc act gtg ctc tcg gac atc atc cag aag tac ttc   4848
Leu Ser Ile Val Gly Thr Val Leu Ser Asp Ile Ile Gln Lys Tyr Phe
              1605                1610                1615

ttc tcc ccg acg ctc ttc cga gtc atc cgc ctg gcc cga ata ggc cgc   4896
Phe Ser Pro Thr Leu Phe Arg Val Ile Arg Leu Ala Arg Ile Gly Arg
          1620                1625                1630
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atc ctc aga ctg atc cga ggg gcc aag ggg atc cgc acg ctg ctc ttt   4944
Ile Leu Arg Leu Ile Arg Gly Ala Lys Gly Ile Arg Thr Leu Leu Phe
      1635                1640                1645

gcc ctc atg atg tcc ctg cct gcc ctc ttc aac atc ggg ctg ctg ctc   4992
Ala Leu Met Met Ser Leu Pro Ala Leu Phe Asn Ile Gly Leu Leu Leu
   1650                1655                1660

ttc ctc gtc atg ttc atc tac tcc atc ttt ggc atg gcc aac ttc gct   5040
Phe Leu Val Met Phe Ile Tyr Ser Ile Phe Gly Met Ala Asn Phe Ala
1665                1670                1675                1680

tat gtc aag tgg gag gct ggc atc gac gac atg ttc aac ttc cag acc   5088
Tyr Val Lys Trp Glu Ala Gly Ile Asp Asp Met Phe Asn Phe Gln Thr
                1685                1690                1695

ttc gcc aac agc atg ctg tgc ctc ttc cag atc acc acg tcg gcc ggc   5136
Phe Ala Asn Ser Met Leu Cys Leu Phe Gln Ile Thr Thr Ser Ala Gly
            1700                1705                1710

tgg gat ggc ctc ctc agc ccc atc ctc aac act ggg ccg ccc tac tgc   5184
Trp Asp Gly Leu Leu Ser Pro Ile Leu Asn Thr Gly Pro Pro Tyr Cys
      1715                1720                1725

gac ccc act ctg ccc aac agc aat ggc tct cgg ggg gac tgc ggg agc   5232
Asp Pro Thr Leu Pro Asn Ser Asn Gly Ser Arg Gly Asp Cys Gly Ser
   1730                1735                1740

cca gcc gtg ggc atc ctc ttc ttc acc acc tac atc atc atc tcc ttc   5280
Pro Ala Val Gly Ile Leu Phe Phe Thr Thr Tyr Ile Ile Ile Ser Phe
1745                1750                1755                1760

ctc atc gtg gtc aac atg tac att gcc atc atc ctg gag aac ttc agc   5328
Leu Ile Val Val Asn Met Tyr Ile Ala Ile Ile Leu Glu Asn Phe Ser
                1765                1770                1775

gtg gcc acg gag gag agc acc gag ccc ctg agt gag gac gac ttc gat   5376
Val Ala Thr Glu Glu Ser Thr Glu Pro Leu Ser Glu Asp Asp Phe Asp
            1780                1785                1790

atg ttc tat gag atc tgg gag aaa ttt gac cca gag gcc act cag ttt   5424
Met Phe Tyr Glu Ile Trp Glu Lys Phe Asp Pro Glu Ala Thr Gln Phe
      1795                1800                1805

att gag tat tcg gtc ctg tct gac ttt gcc gac gcc ctg tct gag cca   5472
Ile Glu Tyr Ser Val Leu Ser Asp Phe Ala Asp Ala Leu Ser Glu Pro
   1810                1815                1820

ctc cgt atc gcc aag ccc aac cag ata agc ctc atc aac atg gac ctg   5520
Leu Arg Ile Ala Lys Pro Asn Gln Ile Ser Leu Ile Asn Met Asp Leu
1825                1830                1835                1840

ccc atg gtg agt ggg gac cgc atc cat tgc atg gac att ctc ttt gcc   5568
Pro Met Val Ser Gly Asp Arg Ile His Cys Met Asp Ile Leu Phe Ala
                1845                1850                1855

ttc acc aaa agg gtc ctg ggg gag tct ggg gag atg gac gcc ctg aag   5616
Phe Thr Lys Arg Val Leu Gly Glu Ser Gly Glu Met Asp Ala Leu Lys
            1860                1865                1870

atc cag atg gag gag aag ttc atg gca gcc aac cca tcc aag atc tcc   5664
Ile Gln Met Glu Glu Lys Phe Met Ala Ala Asn Pro Ser Lys Ile Ser
      1875                1880                1885
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tac gag ccc atc acc acc aca ctc cgg cgc aag cac gaa gag gtg tcg   5712
Tyr Glu Pro Ile Thr Thr Thr Leu Arg Arg Lys His Glu Glu Val Ser
   1890                 1895                 1900

gcc atg gtt atc cag aga gcc ttc cgc agg cac ctg ctg caa cgc tct   5760
Ala Met Val Ile Gln Arg Ala Phe Arg Arg His Leu Leu Gln Arg Ser
1905                1910                 1915                1920

ttg aag cat gcc tcc ttc ctc ttc cgt cag cag gcg ggc agc ggc ctc   5808
Leu Lys His Ala Ser Phe Leu Phe Arg Gln Gln Ala Gly Ser Gly Leu
               1925                 1930                1935

tcc gaa gag gat gcc cct gag cga gag ggc ctc atc gcc tac gtg atg   5856
Ser Glu Glu Asp Ala Pro Glu Arg Glu Gly Leu Ile Ala Tyr Val Met
           1940                 1945                1950

agt gag aac ttc tcc cga ccc ctt ggc cca ccc tcc agc tcc tcc atc   5904
Ser Glu Asn Phe Ser Arg Pro Leu Gly Pro Pro Ser Ser Ser Ser Ile
       1955                 1960                1965

tcc tcc act tcc ttc cca ccc tcc tat gac agt gtc act aga gcc acc   5952
Ser Ser Thr Ser Phe Pro Pro Ser Tyr Asp Ser Val Thr Arg Ala Thr
   1970                 1975                 1980

agc gat aac ctc cag gtg cgg ggg tct gac tac agc cac agt gaa gat   6000
Ser Asp Asn Leu Gln Val Arg Gly Ser Asp Tyr Ser His Ser Glu Asp
1985                1990                 1995                2000

ctc gcc gac ttc ccc cct tct ccg gac agg gac cgt gag tcc atc gtg   6048
Leu Ala Asp Phe Pro Pro Ser Pro Asp Arg Asp Arg Glu Ser Ile Val
               2005                 2010                2015
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             20                  25                  30

Ala Arg Gly Ser Thr Thr Leu Gln Glu Ser Arg Glu Gly Leu Pro Glu
         35                  40                  45

Glu Glu Ala Pro Arg Pro Gln Leu Asp Leu Gln Ala Ser Lys Lys Leu
     50                  55                  60

Pro Asp Leu Tyr Gly Asn Pro Pro Gln Glu Leu Ile Gly Glu Pro Leu
 65                  70                  75                  80

Glu Asp Leu Asp Pro Phe Tyr Ser Thr Gln Lys Thr Phe Ile Val Leu
                 85                  90                  95

Asn Lys Gly Lys Thr Ile Phe Arg Phe Ser Ala Thr Asn Ala Leu Tyr
            100                 105                 110

Val Leu Ser Pro Phe His Pro Val Arg Arg Ala Ala Val Lys Ile Leu
        115                 120                 125
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Val His Ser Leu Phe Asn Met Leu Ile Met Cys Thr Ile Leu Thr Asn
130 135 140

Cys Val Phe Met Ala Gln His Asp Pro Pro Pro Trp Thr Lys Tyr Val
145 150 155 160

Glu Tyr Thr Phe Thr Ala Ile Tyr Thr Phe Glu Ser Leu Val Lys Ile
165 170 175

Leu Ala Arg Ala Phe Cys Leu His Ala Phe Thr Phe Leu Arg Asp Pro
180 185 190

Trp Asn Trp Leu Asp Phe Ser Val Ile Ile Met Ala Tyr Thr Thr Glu
195 200 205

Phe Val Asp Leu Gly Asn Val Ser Ala Leu Arg Thr Phe Arg Val Leu
210 215 220

Arg Ala Leu Lys Thr Ile Ser Val Ile Ser Gly Leu Lys Thr Ile Val
225 230 235 240

Gly Ala Leu Ile Gln Ser Val Lys Lys Leu Ala Asp Val Met Val Leu
245 250 255

Thr Val Phe Cys Leu Ser Val Phe Ala Leu Ile Gly Leu Gln Leu Phe
260 265 270

Met Gly Asn Leu Arg His Lys Cys Val Arg Asn Phe Thr Ala Leu Asn
275 280 285

Gly Thr Asn Gly Ser Val Glu Ala Asp Gly Leu Val Trp Glu Ser Leu
290 295 300

Asp Leu Tyr Leu Ser Asp Pro Glu Asn Tyr Leu Leu Lys Asn Gly Thr
305 310 315 320

Ser Asp Val Leu Leu Cys Gly Asn Ser Ser Asp Ala Gly Thr Cys Pro
325 330 335

Glu Gly Tyr Arg Cys Leu Lys Ala Gly Glu Asn Pro Asp His Gly Tyr
340 345 350

Thr Ser Phe Asp Ser Phe Ala Trp Ala Phe Leu Ala Leu Phe Arg Leu
355 360 365

Met Thr Gln Asp Cys Trp Glu Arg Leu Tyr Gln Gln Thr Leu Arg Ser
370 375 380

Ala Gly Lys Ile Tyr Met Ile Phe Phe Met Leu Val Ile Phe Leu Gly
385 390 395 400

Ser Phe Tyr Leu Val Asn Leu Ile Leu Ala Val Val Ala Met Ala Tyr
405 410 415

Glu Glu Gln Asn Gln Ala Thr Ile Ala Glu Thr Glu Glu Lys Glu Lys
420 425 430

Arg Phe Gln Glu Ala Met Glu Met Leu Lys Lys Glu His Glu Ala Leu
435 440 445

Thr Ile Arg Gly Val Asp Thr Val Ser Arg Ser Ser Leu Glu Met Ser
450 455 460

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Pro Leu Ala Pro Val Asn Ser His Glu Arg Arg Ser Lys Arg Arg Lys
465                 470                 475                 480

Arg Met Ser Ser Gly Thr Glu Glu Cys Gly Glu Asp Arg Leu Pro Lys
                485                 490                 495

Ser Asp Ser Glu Asp Gly Pro Arg Ala Met Asn His Leu Ser Leu Thr
            500                 505                 510

Arg Gly Leu Ser Arg Thr Ser Met Lys Pro Arg Ser Ser Arg Gly Ser
        515                 520                 525

Ile Phe Thr Phe Arg Arg Arg Asp Leu Gly Ser Glu Ala Asp Phe Ala
    530                 535                 540

Asp Asp Glu Asn Ser Thr Ala Arg Glu Ser Glu Ser His His Thr Ser
545                 550                 555                 560

Leu Leu Val Pro Trp Pro Leu Arg Arg Thr Ser Ala Gln Gly Gln Pro
                565                 570                 575

Ser Pro Gly Thr Ser Ala Pro Gly His Ala Leu His Gly Lys Lys Asn
            580                 585                 590

Ser Thr Val Asp Cys Asn Gly Val Val Ser Leu Leu Gly Ala Gly Asp
        595                 600                 605

Pro Glu Ala Thr Ser Pro Gly Ser His Leu Leu Arg Pro Val Met Leu
    610                 615                 620

Glu His Pro Pro Asp Thr Thr Thr Pro Ser Glu Glu Pro Gly Gly Pro
625                 630                 635                 640

Gln Met Leu Thr Ser Gln Ala Pro Cys Val Asp Gly Phe Glu Glu Pro
                645                 650                 655

Gly Ala Arg Gln Arg Ala Leu Ser Ala Val Ser Val Leu Thr Ser Ala
            660                 665                 670

Leu Glu Glu Leu Glu Glu Ser Arg His Lys Cys Pro Pro Cys Trp Asn
        675                 680                 685

Arg Leu Ala Gln Arg Tyr Leu Ile Trp Glu Cys Cys Pro Leu Trp Met
    690                 695                 700

Ser Ile Lys Gln Gly Val Lys Leu Val Val Met Asp Pro Phe Thr Asp
705                 710                 715                 720

Leu Thr Ile Thr Met Cys Ile Val Leu Asn Thr Leu Phe Met Ala Leu
                725                 730                 735

Glu His Tyr Asn Met Thr Ser Glu Phe Glu Glu Met Leu Gln Val Gly
            740                 745                 750

Asn Leu Val Phe Thr Gly Ile Phe Thr Ala Glu Met Thr Phe Lys Ile
        755                 760                 765

Ile Ala Leu Asp Pro Tyr Tyr Tyr Phe Gln Gln Gly Trp Asn Ile Phe
    770                 775                 780

Asp Ser Ile Ile Val Ile Leu Ser Leu Met Glu Leu Gly Leu Ser Arg
785                 790                 795                 800
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805 810 815

Lys Leu Ala Lys Ser Trp Pro Thr Leu Asn Thr Leu Ile Lys Ile Ile
820 825 830

Gly Asn Ser Val Gly Ala Leu Gly Asn Leu Thr Leu Val Leu Ala Ile
835 840 845

Ile Val Phe Ile Phe Ala Val Val Gly Met Gln Leu Phe Gly Lys Asn
850 855 860

Tyr Ser Glu Leu Arg Asp Ser Asp Ser Gly Leu Leu Pro Arg Trp His
865 870 875 880

Met Met Asp Phe Phe His Ala Phe Leu Ile Ile Phe Arg Ile Leu Cys
885 890 895

Gly Glu Trp Ile Glu Thr Met Trp Asp Cys Met Glu Val Ser Gly Gln
900 905 910

Ser Leu Cys Leu Leu Val Phe Leu Leu Val Met Val Ile Gly Asn Leu
915 920 925

Val Val Leu Asn Leu Phe Leu Ala Leu Leu Leu Ser Ser Phe Ser Ala
930 935 940

Asp Asn Leu Thr Ala Pro Asp Glu Asp Arg Glu Met Asn Asn Leu Gln
945 950 955 960

Leu Ala Leu Ala Arg Ile Gln Arg Gly Leu Arg Phe Val Lys Arg Thr
965 970 975

Thr Trp Asp Phe Cys Cys Gly Leu Leu Arg His Arg Pro Gln Lys Pro
980 985 990

Ala Ala Leu Ala Ala Gln Gly Gln Leu Pro Ser Cys Ile Ala Thr Pro
995 1000 1005

Tyr Ser Pro Pro Pro Pro Glu Thr Glu Lys Val Pro Pro Thr Arg Lys
1010 1015 1020

Glu Thr Gln Phe Glu Glu Gly Glu Gln Pro Gly Gln Gly Thr Pro Gly
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Asp Pro Glu Pro Val Cys Val Pro Ile Ala Val Ala Glu Ser Asp Thr
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Asp Asp Gln Glu Glu Asp Glu Glu Asn Ser Leu Gly Thr Glu Glu Glu
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Ser Ser Lys Gln Gln Glu Ser Gln Pro Val Ser Gly Trp Pro Arg Gly
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Pro Pro Asp Ser Arg Thr Trp Ser Gln Val Ser Ala Thr Ala Ser Ser
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Glu Ala Glu Ala Ser Ala Ser Gln Ala Asp Trp Arg Gln Gln Trp Lys
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Ile Val Glu His Ser Trp Phe Glu Thr Phe Ile Ile Phe Met Ile Leu
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Leu Ser Ser Gly Ala Leu Ala Phe Glu Asp Ile Tyr Leu Glu Glu Arg
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Val Ser Leu Val Ser Leu Val Ala Asn Thr Leu Gly Phe Ala Glu Met
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Gly Pro Ile Lys Ser Leu Arg Thr Leu Arg Ala Leu Arg Pro Leu Arg
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Trp Leu Ile Phe Ser Ile Met Gly Val Asn Leu Phe Ala Gly Lys Phe
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Gly Arg Cys Ile Asn Gln Thr Glu Gly Asp Leu Pro Leu Asn Tyr Thr
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Tyr Leu Ala Leu Leu Gln Val Ala Thr Phe Lys Gly Trp Met Asp Ile
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Gly Ser Phe Phe Thr Leu Asn Leu Phe Ile Gly Val Ile Ile Asp Asn
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Phe Asn Gln Gln Lys Lys Lys Leu Gly Gly Gln Asp Ile Phe Met Thr
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Glu Glu Gln Lys Lys Tyr Tyr Asn Ala Met Lys Lys Leu Gly Ser Lys
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Lys Pro Gln Lys Pro Ile Pro Arg Pro Leu Asn Lys Tyr Gln Gly Phe
1505 1510 1515 1520

Ile Phe Asp Ile Val Thr Lys Gln Ala Phe Asp Val Thr Ile Met Phe
1525 1530 1535

Leu Ile Cys Leu Asn Met Val Thr Met Met Val Glu Thr Asp Asp Gln
1540 1545 1550

Ser Pro Glu Lys Ile Asn Ile Leu Ala Lys Ile Asn Leu Leu Phe Val
1555 1560 1565

Ala Ile Phe Thr Gly Glu Cys Ile Val Lys Leu Ala Ala Leu Arg His
1570 1575 1580

Tyr Tyr Phe Thr Asn Ser Trp Asn Ile Phe Asp Phe Val Val Val Ile
1585 1590 1595 1600

Leu Ser Ile Val Gly Thr Val Leu Ser Asp Ile Ile Gln Lys Tyr Phe
1605 1610 1615

Phe Ser Pro Thr Leu Phe Arg Val Ile Arg Leu Ala Arg Ile Gly Arg
1620 1625 1630

Ile Leu Arg Leu Ile Arg Gly Ala Lys Gly Ile Arg Thr Leu Leu Phe
1635 1640 1645

Ala Leu Met Met Ser Leu Pro Ala Leu Phe Asn Ile Gly Leu Leu Leu
1650 1655 1660

Phe Leu Val Met Phe Ile Tyr Ser Ile Phe Gly Met Ala Asn Phe Ala
1665 1670 1675 1680

Tyr Val Lys Trp Glu Ala Gly Ile Asp Asp Met Phe Asn Phe Gln Thr
1685 1690 1695

Phe Ala Asn Ser Met Leu Cys Leu Phe Gln Ile Thr Thr Ser Ala Gly
1700 1705 1710

Trp Asp Gly Leu Leu Ser Pro Ile Leu Asn Thr Gly Pro Pro Tyr Cys
1715 1720 1725

Asp Pro Thr Leu Pro Asn Ser Asn Gly Ser Arg Gly Asp Cys Gly Ser
1730 1735 1740

Pro Ala Val Gly Ile Leu Phe Phe Thr Thr Tyr Ile Ile Ile Ser Phe
1745 1750 1755 1760

Leu Ile Val Val Asn Met Tyr Ile Ala Ile Ile Leu Glu Asn Phe Ser
1765 1770 1775

Val Ala Thr Glu Glu Ser Thr Glu Pro Leu Ser Glu Asp Asp Phe Asp
1780 1785 1790

Met Phe Tyr Glu Ile Trp Glu Lys Phe Asp Pro Glu Ala Thr Gln Phe
1795 1800 1805

Ile Glu Tyr Ser Val Leu Ser Asp Phe Ala Asp Ala Leu Ser Glu Pro
1810 1815 1820

Leu Arg Ile Ala Lys Pro Asn Gln Ile Ser Leu Ile Asn Met Asp Leu
1825 1830 1835 1840

Pro Met Val Ser Gly Asp Arg Ile His Cys Met Asp Ile Leu Phe Ala
1845 1850 1855

Phe Thr Lys Arg Val Leu Gly Glu Ser Gly Glu Met Asp Ala Leu Lys
1860 1865 1870

Ile Gln Met Glu Glu Lys Phe Met Ala Ala Asn Pro Ser Lys Ile Ser
1875 1880 1885

Tyr Glu Pro Ile Thr Thr Thr Leu Arg Arg Lys His Glu Glu Val Ser
1890 1895 1900

Ala Met Val Ile Gln Arg Ala Phe Arg Arg His Leu Leu Gln Arg Ser
1905 1910 1915 1920

Leu Lys His Ala Ser Phe Leu Phe Arg Gln Gln Ala Gly Ser Gly Leu
1925 1930 1935

Ser Glu Glu Asp Ala Pro Glu Arg Glu Gly Leu Ile Ala Tyr Val Met
1940 1945 1950

Ser Glu Asn Phe Ser Arg Pro Leu Gly Pro Pro Ser Ser Ser Ser Ile
1955 1960 1965

Ser Ser Thr Ser Phe Pro Pro Ser Tyr Asp Ser Val Thr Arg Ala Thr
1970 1975 1980

Ser Asp Asn Leu Gln Val Arg Gly Ser Asp Tyr Ser His Ser Glu Asp
1985 1990 1995 2000

Leu Ala Asp Phe Pro Pro Ser Pro Asp Arg Asp Arg Glu Ser Ile Val
2005 2010 2015

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(54) Title: ALTERATIONS IN THE LONG QT SYNDROME GENES *KVLQT1* AND *SCN5A* AND METHODS FOR DETECTING SAME

(57) Abstract: Long QT Syndrome (LQTS) is a cardiovascular disorder characterized by prolongation of the QT interval on electrocardiogram and presence of syncope, seizures and sudden death. Five genes have been implicated in Romano-Ward syndrome, the autosomal dominant form of LQTS. These genes are *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* and *KCNE2*. Mutations in *KVLQT1* and *KCNE1* also cause the Jervell and Lange-Nielsen syndrome, a form of LQTS associated with deafness, a phenotypic abnormality inherited in an autosomal recessive fashion. Mutational analyses were used to screen 262 unrelated individuals with LQTS for mutations in the five defined genes. A total of 134 mutations were observed of which eighty were novel.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/21660

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12Q 1/68; C12P 19/34; C07H 21/04; C07K 14/00, 16/00
US CL : 435/6, 91.1, 91.2; 536/23.1, 24.1; 530/350, 387.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/6, 91.1, 91.2; 536/23.1, 24.1; 530/350, 387.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — Y	FRANQUEZA et al. Long QT Syndrome-associated Mutations in the S4-S5 Linker of KvLQT1 Potassium Channels Modify Gating and Interaction with minK Subunits. Journal of Biological Chemistry. July 23, 1999. Vol. 274, No. 30. pages 21063-21070, see especially abstract, page 21063, fig 1, page 21069.	6 --- 1-5, 7-23,25
Y	NEYROUD et al. Heterozygous Mutation in the Pore of Potassium Channel Gene KvLQT1 Causes an Apparently Normal Phenotype in Long QT Syndrome. European Journal of Human Genetics. 1998, Vol. 6. pages 129-133, see especially abstract, page 129 and 130.	9-11
Y	AN et al. Novel LQT-3 Mutation Affects Na+ Channel Activity Through Interactions Between alpha and beta 1-Subunits. Circulation Research. 1998, Vol. 83, pages 141-146, see especially page 141.	34-36

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
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search terms: KvLQT1, SCN5A, mutation, polymorphism, long qt syndrome, review